THE
HÆMOLYTIC ANÆMIAS
Congenital and Acquired

PART 3

SECONDARY OR SYMPTOMATIC HÆMOLYTIC ANÆMIAS

By

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PREFACE TO THE SECOND EDITION

(PART III)

The completion of this book has taken far longer than was hoped. The reason is simple: the magnitude of the task and the limited time that I have been able to devote to it. Nevertheless, the book has been completed, and in doing so I have, I believe, maintained the standards reached in the first two volumes. It is my hope that the finished book will be looked upon as a reference work of more than ephemeral value.

The final chapters of the book are in fact appearing for convenience of handling in two volumes which are each approximately the same size as Parts I and II.

Part III contains in three chapters accounts of the secondary or symptomatic hæmolytic anæmias and some miscellaneous conditions. Part IV deals, also in three chapters, with the drug-induced hæmolytic anæmias, including favism, paroxysmal nocturnal hæmoglobinuria and hæmolytic disease of the newborn.

I should once more like to record my indebtedness to the many friends who have referred patients to me, and to past and present members of the medical and laboratory staff of the Royal Postgraduate Medical School and Hammersmith Hospital who have helped in the investigation of patients and who have allowed me to quote observations they themselves have made.

I am indebted to the Editor and Publishers of the *British Journal of Hæmatology* for permission to reproduce Figs. 218, 228, 229 and 230, to the Editor of the *British Medical Journal* for permission to reproduce Fig. 242, and to Dr. G. W. Marsh and the Editor of the *Lancet* for permission to reproduce Fig. 243.

I am also greatly indebted to Dr. M. C. Brain and Dr. E. Topley for reading parts of the typescript and for their helpful criticisms and to Dr. S. M. Lewis for valued help in proof-reading. Miss Atkins and her staff in the library at the Royal Postgraduate Medical School have been unfailingly helpful and Mr. W. H. Brackenbury has been similarly helpful and has maintained the high standard of his photomicrographs. Mr. Brecknell and his staff have been of great help in relation to the illustrations. Finally, Miss R. Frearson's accurate typing of the manuscript has been of incalculable value to me, and once again I have received every possible help and encouragement from the publishers.

J. V. Dacie

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CHAPTER 13

SECONDARY OR SYMPTOMATIC HÆMOLYTIC ANÆMIAS: I

HÆMOLYTIC ANÆMIA ASSOCIATED WITH HODGKIN'S DISEASE, LEUKÆMIA, RETICULOSARCOMA AND MYELOSCLEROSIS, ETC.

An accelerated rate of erythrocyte destruction occurs in diseases not primarily affecting erythropoiesis or the erythrocytes. The intensity of hæmolysis* varies from a "silent" degree, only detectable by careful studies of erythrocyte survival, to hæmolysis of sufficient intensity to dominate the clinical picture. In medical literature the terms *secondary* (Watson, 1939) or *symptomatic* (Singer and Dameshek, 1941) have been used to describe the hæmolytic anæmia from which these patients suffer.

In the present Chapter are included descriptions of the hæmolytic anæmias associated with malignant disease of the lymphoreticular system—Hodgkin's disease, chronic lymphocytic leukæmia and reticulosarcoma, as well as macroglobulinæmia, multiple myeloma, miscellaneous types of leukæmia and myelosclerosis. These accounts are followed by descriptions of the hæmolytic anæmias associated with other types of malignant tumour, *e.g.*, disseminated carcinoma and ovarian tumours. In Chapters 14 and 15 will be given descriptions of hæmolytic anæmias associated with a wide range of non-malignant conditions, *e.g.*, collagen diseases, liver disease, infections, renal and vascular disease, etc.

It would have been an ideal arrangement to consider those secondary hæmolytic anæmias which seem clearly to be based on an auto-immune process, as in some cases of chronic lymphocytic leukæmia and reticulosarcoma, and as in systemic (disseminated) lupus erythematosus, etc., in a separate chapter to themselves. As, however, this would have meant dealing with the hæmolytic anæmias of leukæmia, for instance, in two separate chapters, and as the justification for the clear separation of the hæmolytic mechanisms in these cases into auto-immune and non-auto-immune can be argued, this plan has been abandoned in favour of the present arrangement whereby the secondary hæmolytic anæmias, whether apparently auto-immune in origin or not, are considered under the heading of each underlying disease. An idea of the relative frequency of hæmolytic anæmia of the auto-immune type can be obtained from the author's own experience which is summarized in Table 62.

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* The term *hæmolysis* is used here, and in subsequent pages, to denote an increased rate of erythrocyte destruction. It does *not* imply that this is due to any particular mechanism such as amboceptor-complement lysis. *Overt hæmolysis* denotes clinically obvious hæmolysis, as shown by anæmia, jaundice, hæmoglobinuria, etc.; *latent hæmolysis* denotes hæmolysis which is not clinically obvious but which can be demonstrated by measurement of pigment-excretion and/or erythrocyte-survival studies. *Hæmolytic anæmia* is equated with overt hæmolysis. *Excessive* or *excess hæmolysis* means a rate of erythrocyte destruction which is greater than normal, and which may or may not be clinically obvious.

Table 62

Relative Incidence of Different Types of Malignant Reticulosis and Leukæmia Associated with Auto-Immune Hæmolytic Anæmia Postgraduate Medical School of London Data (1947–1965)

Total No. of patients with auto-immune hæmolytic anæmia investigated	250
Total No. of patients with secondary or symptomatic auto-immune hæmolytic anæmia	86
Malignant lymphoma, lymphosarcoma or reticulosarcoma	11*
Plasma cell tumour	1
Chronic lymphocytic leukæmia	11†
Chronic granulocytic leukæmia	0
Other leukæmias	4‡
Hodgkin's disease	2†

* In five of these patients the auto-antibodies were warm-type, and in five they were cold-type. In one patient both warm- and cold-type auto-antibodies were present.

† In each case the auto-antibodies were warm-type.

‡ In three patients the auto-antibodies were warm-type and in one patient cold-type.

In order to avoid too much repetition, the blood pictures, pathogenesis of the hæmolysis and the effects of treament on hæmolysis in the whole group of conditions will be described together. The literature, clinical and serological findings in each disorder will be discussed separately.

HODGKIN'S DISEASE

The association of hæmolytic anæmia and Hodgkin's disease seems to have been first noticed by Netousěk (1922–3) who in an article in a Czech Journal abstracted in *Folia hœmatologica* reported the occurrence of hæmolytic anæmia, and leucopenia, in a patient who died of "lymphogranulomatosis." Sections of the spleen, which had a typical porphyry appearance, showed erythrophagocytosis by macrophages and the accumulation of much yellow pigment, and similar changes were found in the liver and bone marrow. The association of hæmolytic anæmia and Hodgkin's disease was next recognized by Holler and Paschkis (1927) who found that the spleen of a patient suffering from hæmolytic anæmia of unknown origin was infiltrated by lymphadenomatous tissue. Actually, Hedblom (1926) had earlier reported a similar finding at splenectomy in a patient diagnosed as suffering from "splenic anæmia" without recognizing that the anæmia was probably hæmolytic in origin.

Next, Kwaszewska (1931) reported the case of a patient who underwent splenectomy for what was thought to be latent hæmolytic icterus, with microcytosis and increased osmotic fragility, and who subsequently developed enlarged lymph nodes which on biopsy were shown to contain lymphadenomatous tissue. Davidson (1932) described three further examples, and another patient was reported by Schiappoli (1934).

Singer (1936) reported a further case and referred to 11 case reports of a similar nature which he had collected from the literature. von Braitenberg (1937–8) and Bruins Slot (1938) reported further patients and since this time there has been a steady stream of similar case reports (*e.g.*, by Watson, 1939; Marchal, Mahoudeau and Fressinaud, 1940–1; Singer and Dameshek, 1941; Davis, 1944; Grueland, 1947–8; Marchal, 1947 (includes a review of the early literature); Tedeschi and Carnicelli, 1948; Trinick, 1949; Brown and Meynell, 1949; Brown, 1950; Sulzer, 1952; Willcox, 1952; Paraf and Dausset, 1952 (includes a review of the early literature); Foster and Hutt, 1953; Hennemann, 1953; Wassermann *et al.*, 1955; Kyle, Kiely and Stickney, 1959; Molaro and Barillari, 1962; Ranløv and Videbaek, 1963; Hoffbrand, 1964; Hotchkiss, 1964; Matthias, 1964a (includes data on a large series of patients); Payet *et al.*, 1964, and Mandelli *et al.*, 1965 (whose patient was G-6-PD-deficient)).

Clinically, hæmolytic anæmia in Hodgkin's disease presents in several ways. Most commonly, a progressive anæmia develops in an established case: the patient may become slightly jaundiced and examination of his blood reveals a falling hæmoglobin and a raised reticulocyte count. Exacerbations of anæmia commonly parallel periods of increased activity of the disease as shown by pyrexia and by swelling of the lymph nodes and spleen (see Fig. 180 and Ranløv and Videbaek, 1963). Not uncommonly, anæmia of overt hæmolytic type, together with periodic fever, are the presenting symptoms, as in the cases described by Singer (1936) and Dacie (1954, Case 19).

Rather less commonly, hæmolytic anæmia dominates the early clinical picture and only late in the course of the disease, at splenectomy or perhaps at necropsy, is lymph-node enlargement and splenomegaly due to Hodgkin's disease discovered. The cases described by Holler and Paschkis (1927), Kwaszewska (1931), Davidson (1932, 2 cases), Singer (1936), Brown and Meynell (1949) and Wasserman *et al.* (1955) were of the latter type, while in the patient described by Payet and his colleagues (1964) recurrent hæmolytic jaundice preceded by 4 years other clinical manifestations.

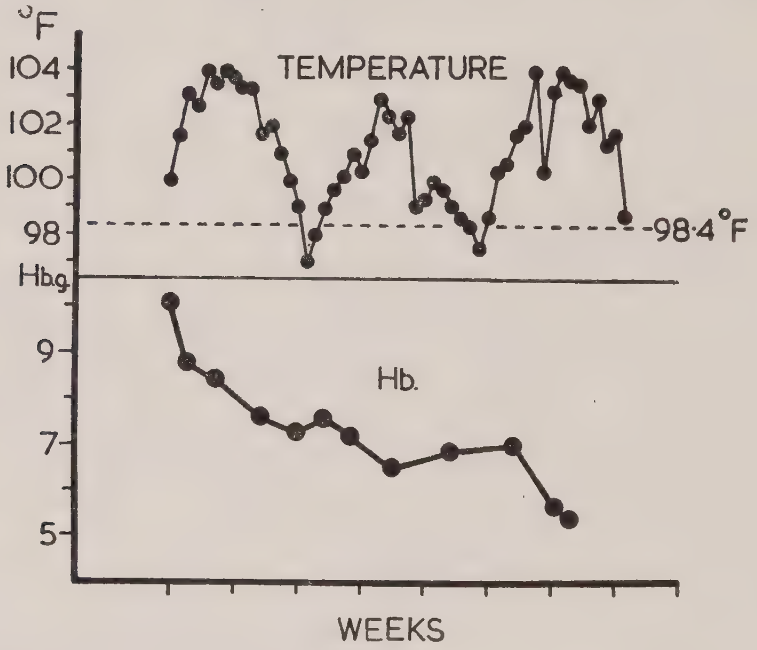

FIG. 180. The relationship between anæmia and pyrexia in a patient suffering from Hodgkin's disease and hæmolytic anæmia. The maximum daily temperature is recorded.

Occasionally, severe anæmia has supervened after a long interval following the successful treatment of locally enlarged lymph nodes without there being any other evidence of recurrence. The remarkable case described by Tedeschi and Carnicelli (1948), whose patient subsequently also developed histological signs of giant follicle lymphoma, and that described by Decker, Rukes and others (1951) were of this type, and another possible instance is that of McFarland, Galbraith and Miale (1960).

A further extraordinary case with a long prodromal illness preceding the advent of histological evidence of Hodgkin's disease was described by Videbaek (1962). This patient's illness commenced with lymph-node enlargement, plasmacytosis in the bone marrow and peripheral blood, hypergammaglobulinæmia and anæmia and marrow hypoplasia. Later the anæmia was accompanied by reticulocytosis. Still later, Hodgkin's disease was diagnosed as the result of lymph-node biopsy.

Incidence of Hæmolytic Anæmia

Although most patients suffering from Hodgkin's disease become anæmic in the course of their illness, overt hæmolytic anæmia is not common. Wasserman and his colleagues (1955) reported, for instance, the occurrence of eight such cases in 104 consecutive patients. However, there is little doubt, if the criterion for hæmolytic anæmia was based on the finding of a demonstrable shortening of the erythrocyte life-span (see below), that the incidence of increased hæmolysis would be far greater, particularly in patients in the terminal stages of their disease. Matthias (1964a) concluded in fact that almost all patients suffer from increased hæmolysis at this time.

Erythrocyte Survival Data. Most of the available data have demonstrated a slight to moderate, and in a few cases a marked, degree of hæmolysis.

Jensen (1957) studied ten cases: the ^{51}Cr T$\frac{1}{2}$ ranged from 13 to 28 days, being shortened in eight of the patients. There appeared, however, to be no correlation between the size of the spleen and hæmolysis. The reticulocyte counts were normal, except in one case, and none of the patients gave a positive direct antiglobulin test. Waggener, Pratt and Hunt (1958) studied five patients: their ^{51}Cr T$\frac{1}{2}$ ranged from 15 to 23 days, with a mean of 19 days. Giannopoulos and Bergsagel (1959) investigated six patients, all anæmic and pyrexial: the ^{51}Cr T$\frac{1}{2}$ was reduced in each case, varying from 9·5 to 22 days with a mean of 16·7 days. Finally, Veeger and colleagues (1962) reported on six patients: their ^{51}Cr T$\frac{1}{2}$ ranged from 2·8 to 21 days and in four out of five patients there was evidence of accumulation of radioactivity in the spleen. Four of these patients underwent splenectomy. The erythrocyte survival lengthened in each case and in two of them became normal. (It is interesting to note that one of these patients was not considered to show evidence of erythrocyte sequestration in the spleen pre-operatively.)

Serological Findings

In most cases of Hodgkin's disease, even when there is overt hæmolysis, tests for auto-antibodies give negative results. Thus of 31 cases of auto-immune hæmolytic anæmia associated with malignant reticuloses or leukæmia studied by the author there were only two cases of Hodgkin's disease* (Table 62; see also Case 14). The literature by now, however, contains reports of a moderate number of such cases, most of which are referred to below.

Trinick (1949) seems to have been the first to mention, in a brief report, the finding of a positive direct antiglobulin test. A similar observation, with fuller details, was recorded by Rundles and Barton (1952). Auto-agglutination, was noted and the patient's serum was thought to contain a panagglutinin. Additional cases were described by Hennemann (1955–6)—one positive antiglobulin test in 52 patients—and Wasserman, Stats, Schwartz and Fudenberg (1955)—two positive antiglobulin tests in eight cases of hæmolytic anæmia out of a total of 104 consecutive patients with Hodgkin's disease. Ninni (1956) recorded two further cases. Grobbelaar (1958) reported three positive tests in seven patients studied, and noted both warm-type and cold-type antiglobulin reactions. Kyle, Kiely and Stickney (1959) observed positive antiglobulin tests in three out of seven patients, all of whom were severely anæmic.

Other patients giving positive antiglobulin reactions have recently been described by McFarland, Galbraith and Miale (1960) (1 case), Bousser and his colleagues (1961) (4 cases) and Videbaek (1962) (2 cases). The serum of the patient described by Hougie, Dandridge and Bobbitt (1959) contained an unusual antibody; it had no action on fresh erythrocytes but agglutinated all the cells tested (including the patient's own cells) if they had been first stored for 48 hours or more. However, if papainized, fresh cells were strongly agglutinated.

More recently, Matthias (1964a), in a comprehensive study, has reported a relatively high incidence of positive direct antiglobulin reactions—namely, 12 positives in 50 patients (24%); the antiglobulin titre was greater than 64 in six.

A brief account of one of the two patients studied by the author in whom Hodgkin's disease appeared to be complicated by an auto-immune hæmolytic anæmia is given below (Case 14).

Case Report: Auto-Immune Hæmolytic Anæmia Associated with Hodgkin's Disease

Case 14. Mrs. C. E., aged 43, had suffered from increasing malaise over a period of 2 years. In May 1964, 6 months before admission into Hammersmith Hospital, she became short of breath and was admitted into another hospital where she was found to be anæmic and jaundiced. The spleen and liver were enlarged and she had signs of thrombophlebitis in the right leg. The direct antiglobulin test was then weakly positive; there was marked spherocytosis and a reticulocyte count of 60%. She was transfused and splenectomy was carried out.

Sections of the spleen showed a normal architecture. The Malpighian bodies were slightly larger than normal and they had active germinal centres. The spleen pulp and its reticulin content and pattern appeared normal. Sections of an abdominal lymph node (2·3 × 1·5 cm.), taken at the time of splenectomy, showed the changes of Hodgkin's disease (Prof. C. V. Harrison). The structure was completely destroyed: the reticulin pattern was irregular and normal cells were replaced by a mixture of lymphocytes and primitive reticulum cells amongst which were many Sternberg-Reed giant cells.

---

* The author unfortunately has no data on the proportion of cases of Hodgkin's disease, with or without clear evidence of hæmolysis, in which the antiglobulin test becomes positive. Reliable figures could not in any case be obtained without carrying out repeated tests on a large series of patients followed to the end of their illness, which is an almost impossible task.

The patient remained well for a few weeks after the operation, but then excessive fatigue and generalized aches and pains returned, and she again became jaundiced; symptoms of thrombophlebitis also recurred. She was found to be severely anæmic, and in November 1964 she was transferred to Hammersmith Hospital.

Physical Examination. She was an ill-looking woman and was mentally confused and disorientated; she was markedly pale and also jaundiced. Her temperature was 100° F.

The liver was palpable 8 cm. below the costal margin, but no enlarged lymph nodes could be felt. The right calf was tender and swollen.

Laboratory Investigations. The blood count on admission was as follows: erythrocytes 0·6 million per cu. mm.; hæmoglobin 3·3 g. per 100 ml.; PCV 14%; reticulocytes 90%; leucocyte count 27,000 per cu. mm., with 62% neutrophils, 2% metamyelocytes, 1% eosinophils, 25% lymphocytes and 10% monocytes; platelet count 20,000 per cu. mm. Blood films showed marked anisocytosis with macrocytosis, polychromasia, anisochromasia and microspherocytosis. Howell-Jolly and Pappenheimer bodies were present, and these were 120 erythroblasts per 100 leucocytes (Fig. 182).

The serum bilirubin was 8·1 mg. per 100 ml., serum iron 264 μg. per 100 ml. and serum urea 68 mg. per 100 ml. Total serum proteins were 7·1 g. per 100 ml. (albumin 4·1 g. and globulins 3·0 g.). The serum B_{12} was 160μμg. per ml. and serum folate 3·0 μmg. per ml. (subnormal).

The urine contained a trace of albumin; the urine deposit was normal and no hæmosiderin was demonstrated.

Bone-marrow aspiration yielded a hypercellular marrow. Erythropoiesis was very active; it was normoblastic in type. An excess of iron was present in the marrow fragments; siderotic granules were unduly prominent in erythroblasts and a few ring-type sideroblasts were present.

Serology. The direct antiglobulin test was strongly positive, both with anti-γG and anti-non-γ (anti-C′) sera. The cold-agglutinin titre was 16 at 4° C. and 1 at 20° C. Antibody active at 37° C. could be detected by enzyme-treated (papainized) cells: the agglutinin titre was 16 and the hæmolysin titre 4. The indirect antiglobulin test was, however, negative. Serum complement activity was reduced (12 units). An eluate prepared from the patient's erythrocytes did not appear to have any specificity. The patient's blood group was A Rh-positive. The above findings were interpreted as indicating the formation of a "non-specific" warm (γG) anti-erythrocyte antibody which had some complement-fixing ability. Antibody free in the serum could only be demonstrated by the use of enzyme-treated cells.

Course and Treatment. The patient was treated immediately with digoxin, prednisone and ACTH and she was transfused with the packed cells derived from 2 l. of blood (Fig. 181). Her symptoms responded well to this treatment but she then developed signs of congestive cardiac failure. This in turn was treated successfully.

The day following admission she developed signs of deep vein thrombosis in the left leg and she also complained of central chest pain. An ECG (and a striking rise in serum lactic dehydrogenase) confirmed the diagnosis of myocardial infarction. Anticoagulant therapy was commenced, and the œdema responded to diuretic therapy.

Subsequently, the blood picture improved strikingly (Fig. 181), the hæmoglobin reaching a peak value of 14·8 g. per 100 ml. 25 days after admission. The ACTH and prednisone were in due course stopped and reduced to 12·5 mg. daily, respectively. However, within 2–3 weeks severe hæmolysis recurred which could not be controlled by the use of ACTH and prednisone. Chest pain, attributed to pulmonary emboli, also developed. She died on the 24th December 1964.

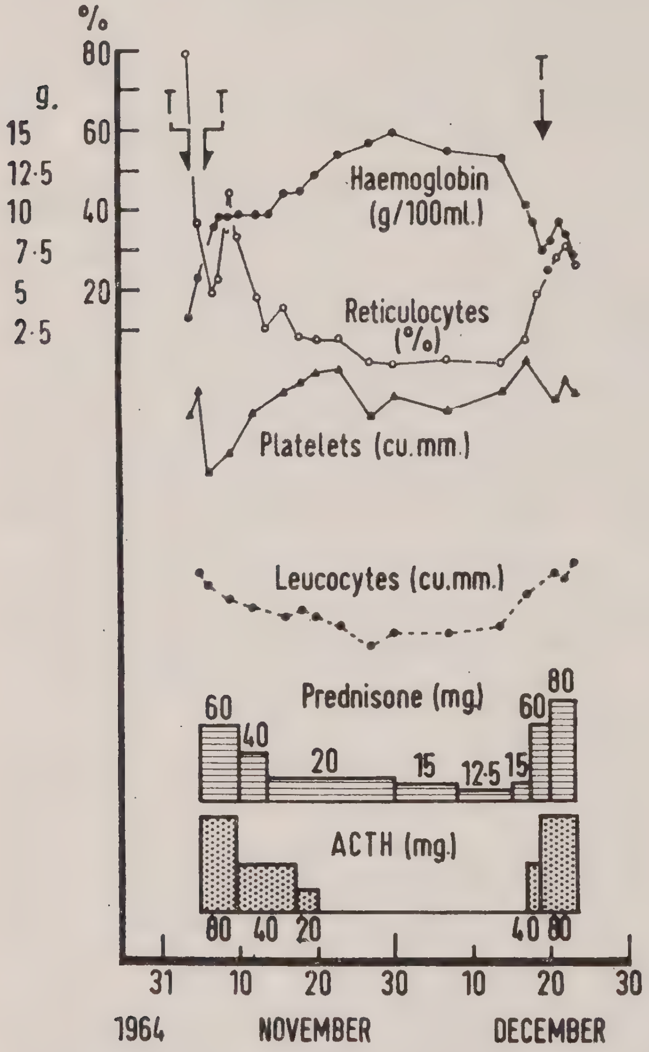

Fig. 181. Hæmatological changes in a patient suffering from Hodgkin's disease and auto-immune hæmolytic anæmia (Case 14). Response to ACTH and adrenocorticosteroid therapy.
T = transfusions.

Post-mortem examination showed the signs of severe anæmia, deep vein thrombosis in both legs, pulmonary embolism and the presence of deposits of Hodgkin's disease, particularly in abdominal lymph nodes. Sections of these nodes showed that the tumour had become transformed into a pleomorphic reticulosarcoma (Hodgkin's sarcoma).

Summary. A case of Hodgkin's disease presenting as an auto-immune hæmolytic anæmia of warm-antibody type. The diagnosis was made from a lymph-node biopsy taken at the time of splenectomy. Death was due to pulmonary embolism and uncontrolled anæmia.

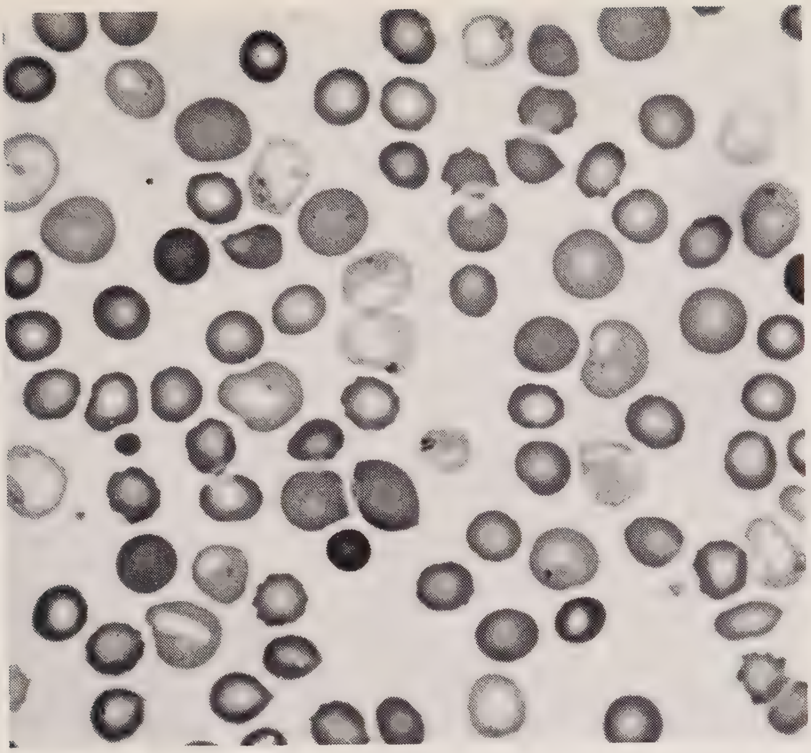

FIG. 182. Photomicrograph of a blood film of a patient suffering from Hodgkin's disease complicated by an auto-immune hæmolytic anæmia (Case 14). There is considerable anisocytosis, macrocytosis, microspherocytosis and anisochromasia. Pappenheimer bodies are conspicuous. (The patient had been splenectomized.) × 700.

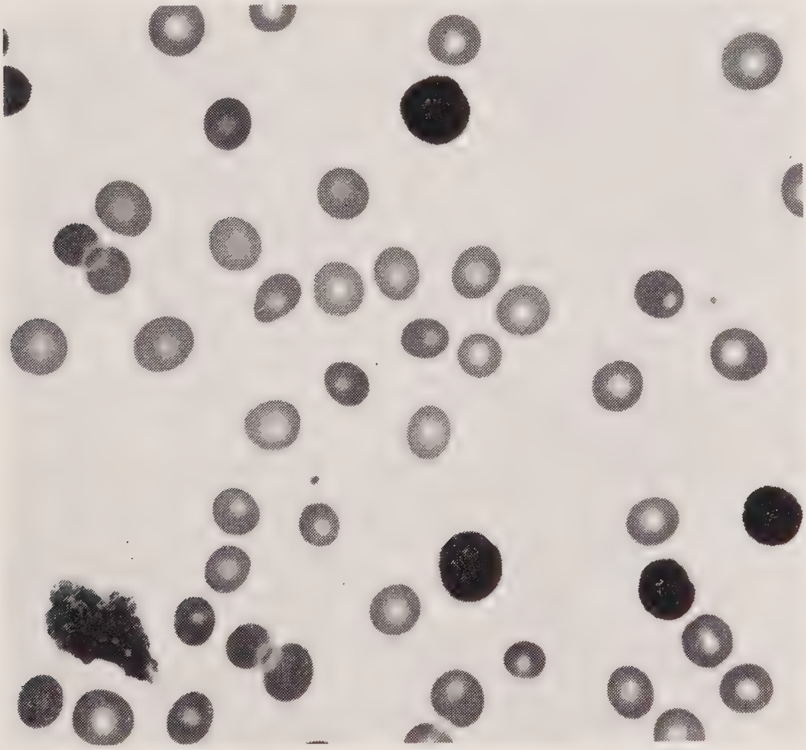

FIG. 183. Photomicrograph of a blood film of a patient suffering from chronic lymphocytic leukæmia and auto-immune hæmolytic anæmia (Case 15). There is slight anisocytosis and microspherocytosis. × 700.

LEUKÆMIA

The possible role of hæmolysis in the pathogenesis of anæmia in leukæmia was referred to by Hirschfeld and by Pappenheim as long ago as 1906 in a discussion on a case of "leukanæmia". Subsequently, many other instances have been described in which excess hæmolysis was thought to be occurring or, in recent years, has been proved to be occurring by actual measurements of erythrocyte survival. It is probably true to say that an increased rate of hæmolysis contributes to the anæmia in most, if not in all, cases of leukæmia at some stage in the evolution of the disease. This probably applies to all types of leukæmia.

An early case report of exceptional interest was that of Rowley (1908). This report dealt with a patient whose total leucocyte count was 800,000 per cu.mm. The majority of the leucocytes were stated to be myeloid in origin, and many of them could be seen to be phagocytosing all types of leucocytes as well as erythrocytes. Hæmolysis must have played a part in this patient's anæmia.

Other early descriptions in which excessive hæmolysis was considered to be present include the following: Tixier and Troisier (1910)—a case of chronic granulocytic leukæmia with increased erythrocyte osmotic fragility, slight microcytosis and jaundice; Brill (1924)—a case of subacute granulocytic leukæmia; Alexander and Thompson (1925)—a case of doubtful cytological type associated with the cold-hæmagglutinin syndrome; Paschkis (1927)—cases of chronic granulocytic leukæmia and lymphocytic leukæmia, respectively; and Jaffé (1935)—cases of acute leukæmia.

Studies of pigment metabolism have also pointed to excessive hæmolysis in certain patients. Barker (1938) studied urobilinogen excretion in nine patients with leukæmia and found that the amount of fæcal pigment was definitely raised in two of them (suffering from myeloid leukæmia); their reticulocyte counts were 7·6% and 6·2%, respectively. A higher frequency of excess hæmolysis was suggested by the data of Morelli and D'Ambrosio (1939), which was based on a study on 19 patients with chronic or subacute lymphocytic or granulocytic leukæmia. They observed an increased urobilinogen output and a raised hæmolytic index in many of them and concluded that their anæmia was in part due to hæmolysis within the spleen.

A review of the early literature on the occurrence of overt hæmolysis in leukæmia was published by Paraf and Dausset (1952); more recent references were given by Price and Greenfield (1958).

In general, as is also true of Hodgkin's disease, hæmolysis is most rapid and obvious in the terminal stages of leukæmia, but there are exceptions to this. In chronic lymphocytic leukæmia, in particular, an auto-immune hæmolytic anæmia quite frequently develops and this may dominate the clinical picture. This is, however, rare in other types of leukæmia. Some recent papers on hæmolysis in the different types of leukæmia are summarized in the following sections.

RECENT LITERATURE ON EXCESSIVE HÆMOLYSIS IN LEUKÆMIA

Chronic Granulocytic Leukæmia

Marchal and Duhamel (1950) referred to three cases of myeloid leukæmia in which hæmolytic anæmia was thought to be present and Jonsson, Hansen-Pruss and Rundles (1950) described a patient who suffered from myeloid leukæmia and whose blood transfusion requirement was greatly reduced by splenectomy. A rather unusual case in which pseudo-Pelger cells were present and in which there was also some evidence of hæmolysis was reported by Darte, Dacie and McSorley (1954).

The work of Berlin (1951), who employed the Ashby method of differential agglutination, suggested that latent, as opposed to overt, hæmolysis was frequently present in chronic granulocytic leukæmia. Studying the fate of transfused normal erythrocytes, he found evidence of reduced survival in 12 out of 15 patients; in seven (mostly with large spleens) hæmolysis was in fact quite markedly increased, 50% of the transfused cells being eliminated within 10 days or less. Most of these patients had raised reticulocyte counts (2·4–9·4%). Erythrocyte osmotic fragility was definitely increased in two patients.

Böttner and Schlegel (1952), as the result of studies carried out by the Ashby method on 28 patients, concluded that the survival of transfused normal erythrocytes was frequently shortened in both acute and chronic leukæmia, including chronic granulocytic leukæmia, as well as in a variety of types of lymphoma.

N.I.Berlin, Lawrence and Lee (1954), by employing ^{14}C-labelled glycine, were able to study the life-span of the patients' own erythrocytes: in five cases of chronic granulocytic leukæmia the mean life-span was computed to be between 70 and 100 days, a slight shortening. Verloop (1955) referred to two patients out of a total of seven studied whose fæcal urobilinogen excretion was increased.

Further studies of erythrocyte survival have been carried out in cases of chronic granulocytic leukæmia by labelling the patients' own cells with ^{51}Cr (Wetherley-Mein, Epstein, Foster and Grimes, 1958; Tudhope, 1959). The survival was impaired in a proportion of the patients studied in each series. Bowdler and Prankerd (1962) reported reduced survival in three patients and Pengelly and Wilkinson (1962) normal survival in five patients. Eernisse and van Rood (1961), who used DF^{32}P, reported a shortened survival in one patient.

It is difficult to know the exact incidence of increased hæmolysis in chronic granulocytic leukæmia. Minor degrees of hæmolysis are probably frequent at least in the terminal stages of the disease. Major degrees are clearly rare, and in this respect chronic granulocytic leukæmia differs from chronic lymphocytic leukæmia in which clear evidence of hæmolysis is not uncommon.

Serological Findings. The direct antiglobulin test has seldom been reported as positive in chronic granulocytic leukæmia. An idea of the incidence of positive tests is given by Hennemann (1955–6)—one positive reaction in 25 cases, and by Videbaek (1962)—one moderately strong and one weak positive reaction in two patients with overt hæmolytic anæmia out of a total of 27 consecutive cases. The author's own experience underlines the rarity of an auto-immune hæmolytic mechanism in chronic granulocytic leukæmia: out of a total of 250 cases of "idiopathic" or secondary auto-immune hæmolytic anæmia investigated there was not a single example of chronic granulocytic leukæmia (Table 62).

An interesting but unusual case was described by Krug (1956). This seems to have been a typical example of chronic granulocytic leukæmia except that at necropsy the marrow showed a marked "reticulosis" [? increase in reticulin] as well as containing many unripe granulopoietic cells. The direct antiglobulin test was positive.

Chronic Lymphocytic Leukæmia

The occurrence of hæmolytic anæmia in association with chronic lymphocytic leukæmia is exceptionally well documented. As already referred to, a probable instance of this was reported by Paschkis in 1927. Other early records include those of Klima (1934–5), Klima and Seyfried (1937), Haden (1939), Singer and Dameshek (1941) (two cases), Feuchtinger (1942), Feldman and Yarvis (1944)—an exceptionally interesting case in which there was suggestive evidence, too, of infectious mononucleosis, and Bøe (1946).

Later, cases began to be reported in which the direct antiglobulin test was positive and it was not long before it was realized that the majority of the most severe and obvious examples of hæmolytic anæmia occurring in the course of lymphocytic leukæmia were of the auto-immune type, with positive direct antiglobulin tests and sometimes, too, with auto-antibody free in the patient's serum. Cases of this type have thus been reported by Trinick (1949); Jordan and Dingle (1949); Rosenfield, Vogel and Rosenthal (1951); Dameshek, Rosenthal and Schwartz (1951); Willcox (1952); Craig, Waterhouse and Young (1952); Hennemann, Falck and Gillert (1953); Rosenthal and co-workers (1955); Chanarin, Dacie and Mollin (1959); Pisciotta, Jermain and Hinz (1960); Videbaek (1962); and Ebbe, Wittels and Dameshek (1962). Many more examples have been published in recent years. Most of these studies have been concerned with serological niceties, measurements of erythrocytic life-span, responses to treatment, and pathogenesis. (Some of these papers will be referred to when these topics are discussed.) The incidence of auto-immune hæmolytic anæmia will, however, be dealt with at this point, and some clinical details referred to.

Incidence of Auto-Immune Hæmolytic Anæmia in Chronic Lymphocytic Leukæmia

The reported incidences vary from 10 to 25%, approximately, but not all authors have clearly distinguished between those cases which gave positive antiglobulin tests and those which did not.

Verloop (1955) reported on 15 cases of chronic lymphocytic leukæmia; fæcal urobilinogen excretion was raised in about half of them and the direct antiglobulin test was positive in two. Hennemann (1955–6) referred to 24 cases: there were two clear instances of hæmolytic anæmia and one doubtful, and the antiglobulin test was positive in one case. Seaman, Koler, Pirofsky and Osgood (1958) reported 50 instances of severe hæmolytic anæmia in 200 consecutive patients (25%), but without attempting to discriminate between the cases which appeared to be auto-immune and those which did not. The time of onset of overt hæmolysis in this large series ranged from before the diagnosis of the leukæmia to 150 months after its onset; the mean time of onset was 54 months from diagnosis and the development of the complication was thought to be a random event.

Dameshek and Gunz (1964a) gave the overall incidence of auto-immune hæmolytic anæmia as about 20%. They also mentioned the occurrence of occult hæmolytic disease, with a negative antiglobulin test, in the terminal phases of the illness, but they stressed the apparent independence of overt hæmolytic anæmia and the underlying disease. Beikert (1959) reviewed 19 cases of chronic lymphocytic leukæmia and found that three had overt hæmolytic anæmia, while Bousser and Pean (1959) reported that nine out of 21 patients showed signs of hæmolysis.

Troup, Swisher and Young (1960) stated that ten out of 77 cases of chronic lymphocytic leukæmia developed auto-immune hæmolytic anæmia. Videbaek's (1962) figures were 12 out of 47 consecutive cases. The incidence in Galton's (1963) large series has been less, but antiglobulin tests were only carried out on patients who showed signs of overt hæmolytic anæmia. There were 17 such patients out of a total of 88, but only four of the 17 gave positive antiglobulin tests.

Matthias (1964a) reported that ten out of 50 patients (20%) suffering from lymphoproliferative disease gave positive antiglobulin tests; in six the antiglobulin titre exceeded 64. However, he made the point that positive tests were often transient and stated that in only three of his patients with chronic lymphocytic leukæmia was the test persistently positive.

It is difficult to arrive at even an approximate incidence for the occurrence of excessive hæmolysis from the available data. Clearly, all patients should be followed to the end of their illness before it can be stated categorically whether they did or did not develop clear signs of hæmolysis or a positive antiglobulin test, and in none of the above-quoted series does it seem that it was possible to do this. In relation to the incidence of positive antiglobulin tests it would be necessary, too, to test all patients, irrespective of whether they showed signs of obvious hæmolysis, and this, too, has not always been done. Again, unless cell survival studies were done repeatedly throughout a patient's illness, a manifestly impracticable task, it would not be possible to say, in many cases, whether there was increased hæmolysis or not; and the margin between occult and overt hæmolysis is, too, necessarily ill-defined and open to argument unless based on actual measurements of cell survival.

As already stated, the occurrence of overt hæmolytic anæmia, in particular of the auto-immune type, is capricious; its onset may antedate the signs of lymphocytic leukæmia (Rosenthal *et al.*, 1955) or it may occur many months or years after its onset. Amongst the patients reported by Rosenthal and his colleagues (1955) there were in fact nine in whom lymphocytic leukæmia was discovered only when the onset of hæmolytic anæmia brought the patient to hospital. They remarked on the unusually large size of the spleen in the patients suffering from hæmolytic anæmia. Videbaek (1962) gave further clinical details. In his series, too, overt hæmolytic anæmia was in some cases the first sign of illness, but, at the other extreme, it did not occur in one patient until 12 years after the onset of the leukæmia. Videbaek pointed out that hæmolytic episodes could be transient, lasting only 2–3 weeks, or be continuous. Usually there was only one phase of hæmolysis, but there might be as many as two or three. In these respects, and in serology, too (see below), the auto-immune hæmolytic anæmia of lymphocytic leukæmia does not differ from the "idiopathic" disease. Equally interesting is the occurrence in chronic lymphocytic leukæmia of thrombocytopenic purpura in association with auto-immune hæmolytic anæmia (Ebbe, Wittels and Dameshek, 1962); this also occurs in cases without the background of leukæmia.

Measurements of Erythrocyte Life-Span. Trinick (1949) reported the "rapid" elimination of transfused normal erythrocytes in one case, and Brown, Elliott and Young (1951) moderately increased, slightly increased or normal survival in three cases, respectively. These authors employed the Ashby technique. Weinstein and LeRoy (1953) used ^{51}Cr and found a normal ^{51}Cr $T\frac{1}{2}$ in one case, a slight shortening (23 days) in another and a marked shortening in a third (9 days). Read, Wilson and Gardner (1954) reported a very short survival (^{51}Cr $T\frac{1}{2}$ 2·5 days) in one case. Berlin, Lawrence and Lee (1954) used ^{14}C-glycine and found a marked shortening of erythrocyte life-span in one case but normal results in two others.

More recent reports of measurements using ^{51}Cr include those of Jensen (1957), Ultmann (1958), Ferymann, Burrell and Marler (1958), Tudhope (1959) and Pengelly and Wilkinson (1962). The results of these studies have confirmed those of the earlier work cited above: namely, that survival may be markedly shortened—usually in cases giving a positive antiglobulin test—moderately impaired or strictly normal. Ultmann (1958), who studied patients in whom the antiglobulin test was negative, and who carried out surface counting of radioactivity over various organs, found, despite lack of evidence of an auto-immune process, a moderate to marked increase in the splenic uptake of chromium in several cases.

Serological Studies. The nature of the protein giving a positive antiglobulin test in cases of chronic lymphocytic leukæmia has been the subject of much discussion. More than one type of "auto-antibody" may be involved.

Rosenthal and his co-workers (1955) emphasized the similarity between the serological reactions of erythrocytes and serum antibodies in "idiopathic" auto-immune hæmolytic anæmia and those found in chronic lymphocytic leukæmia. The author's own data, summarized in Table 63, point in the same direction and, in relation to specificity, Gold, Ikin and Mourant (1958) were able to demonstrate in one case three specific Rh auto-antibodies, anti-c, anti-D and anti-e.

Pisciotta, Jermain and Hinz (1960) demonstrated cross-reactivity between the protein coating their patients' erythrocytes and antiglobulin sera prepared by injection of either eluted antibodies derived from patients with "idiopathic" auto-immune hæmolytic anæmia or of normal γ globulin. They showed, too, that ^{131}I-labelled normal γ globulin was not adsorbed *in vivo* to the

Table 63

Serological Findings in Auto-Immune Hæmolytic Anæmia Associated with Chronic Lymphocytic Leukæmia (Worlledge and Dacie, unpublished data)

Initials of patient	Sex Age	Antiglobulin test			Agglutination			Lysis				Type of auto-antibody	Specificity of auto-antibody
		Direct		Indirect 37° C.	Normal cells (titre)		Papainized normal cells (titre) 37° C.	PNH cells 37° C.	Papainized PNH cells 37° C.	Papainized normal cells			
		Anti-γG	Anti-non-γ		2–4° C.	20° C.				Stored 37° C.	Fresh 37° C.		
A.F.	M.71	+ + +	—	+	...	< 1	+	—	—	—	—	Warm	Anti-e + “NS”
A.B.	M.57	+ +	—	+	< 1	< 1	64	—	—	—	—	Warm	“NS”
E.B.	F.73	+ + + +	—	+	16	< 1	1,024	—	+ +	+	—	Warm	“NS”
E.T.	M.77	+ +	—	—	< 1	< 1	< 1	—	—	—	—	Warm	“NS”
A.S.	F.51	+ + +	+ +	—	< 1	< 1	16	...	...	...	...	Warm	?“NS”
W.S.	M.58	+ +	+	+	< 1	< 1	16	...	...	...	...	Warm	“NS”
H.D. (Case 15)	M.65	+ + +	+	+ +	< 1	< 1	16	—	—	—	—	Warm	“NS”
F.B.	F.68	+ + +	—	—	...	...	1	—	—	—	—	Warm	“NS”
W.W.	M.72	+ + +	—	—	...	< 1	< 1	—	—	—	—	Warm	“NS”
K.K.	M.53	+ +	—	—	< 1	< 1	< 1	—	—	—	—	Warm	...
F.S.	M.65	—	+ ±	—	...	< 1	1	...	...	±	—	Warm	“NS”

+ + + + denotes strong naked-eye agglutination or hæmolysis; + + +, + +, + ±, + and ± denote lesser degrees of agglutination or hæmolysis; ... denotes no observation.

“NS” denotes no specificity demonstrated.

patient's erythrocytes and concluded that the coating material was of endogenous nature. Chaplin and Cassell (1960) and Cassell and Chaplin (1960) in a more comprehensive study demonstrated the broad cross-reactivity between antiglobulin sera prepared, respectively, against antibodies eluted from erythrocytes from cases of either "idiopathic" or secondary auto-immune hæmolytic anæmia and conventional antiglobulin sera.

The possibility that the circulating abnormal lymphocytes might also take part in serological reactions was investigated by Pirofsky (1956); he failed, however, to demonstrate any reaction between various types of leucocyte extract and eluted antibodies derived from the erythrocytes of four patients suffering from chronic lymphocytic leukaemia and hæmolytic anæmia. Beikert (1959), on the other hand, described and illustrated how leukæmic lymphocytes, derived from a patient whose illness was accompanied by hæmolysis, were agglutinated in antiglobulin serum although lymphocytes from two cases in which there were no signs of hæmolysis remained unagglutinated. This observation led him to suggest that the immune bodies that could be demonstrated in cases of chronic lymphocytic leukæmia with auto-immune hæmolytic anæmia might be auto-antibodies directed against degenerate neoplastic lymphocytes, which also had the ability to cross-react with erythrocytes.

Brody has more recently investigated this point in detail. First, Brody and Finch (1961) reported that they had failed to demonstrate positive immune adherence (using guinea-pig platelets) and concluded that the serum factors which coated erythrocytes of patients with lymphomas might be manifestations of a dysproteinæmic state rather than auto-antibodies. Later, however, Brody and Chertok (1962) found that the immune adherence test was positive when eluates from erythrocytes were allowed to react with autologous leukæmic lymphocytes—the tests were negative with autologous erythrocytes. They suspected that the leukæmic lymphocytes might act as antigen in the development of the erythrocyte-coating globulin. Subsequently, Brody and Beizer (1963) used a mixed-cell agglutination technique and showed that when the antiglobulin test on erythrocytes was positive the lymphocytes, too, were coated with globulin. It appeared also that the globulin coating the lymphocytes might dissociate and coat erythrocytes.

The interesting observations described above do not fit in with the concept that the erythrocyte-coating material is essentially of a similar nature in cases of auto-immune hæmolytic anæmia accompanying chronic lymphocytic leukæmia and in "idiopathic" cases. It is, however, possible that Brody and his colleagues were not investigating "typical" cases: for one thing, the direct antiglobulin test was maximally positive in undiluted antiglobulin serum and the maximum serum dilution giving positive agglutination was 1 in 48. These reactions are unlike most of those seen by the author in patients with lymphocytic leukæmia accompanied by active hæmolysis or those produced when the coating material is γG(7S) globulin.

A brief account of a patient personally studied by the author follows (Case 15).

Case Report: Auto-Immune Hæmolytic Anæmia Complicating Chronic Lymphocytic Leukæmia

Case 15. The patient, H. D., was a male aged 65. He had been treated at a Chest Clinic for a number of years for bronchitis. In January 1959 he complained of increasing breathlessness and was found to be anæmic and to have a large spleen. He was admitted into hospital and transfused. A diagnosis of chronic lymphocytic leukæmia was made and he was referred in March 1959 to Hammersmith Hospital for radiotherapy.

Physical Examination. His general condition was found to be poor and he was markedly breathless on exertion. He was pale but not obviously jaundiced. The spleen was palpable 12 cm. below the left costal margin and the liver palpable 3 cm. below the right. Small enlarged lymph nodes were felt in both axillæ.

Laboratory Findings. On admission the hæmoglobin was 5·7 g. per 100 ml.; the PCV was 17% and reticulocyte count <0·1%. The leucocyte count was 193,000 per cu. mm., with 5% neutrophils and 95% lymphocytes. The platelet count was 214,000 per cu. mm.

Stained films showed numerous small lymphocytes with scanty cytoplasm and a moderate amount of erythrocyte anisocytosis. Some spherocytes were present but no macrocytosis or polychromasia (Fig. 183).

The erythrocyte osmotic fragility was slightly increased (MCF 0·48% NaCl). Bone-marrow aspiration provided no fragments of marrow, the material obtained consisting almost entirely of a suspension of lymphocytes. Only a few normoblasts were seen. The serum-bilirubin was 0·8 mg. per 100 ml. The urine was normal.

A ^{51}Cr auto-survival was started soon after admission. This showed a shortened erythrocyte life-span (mean cell life-span 10 days) and some excess accumulation of radioactivity in the spleen.

Serology. The direct antiglobulin test was strongly positive, + + + with anti-γG serum and + with anti-non-γ (anti-C′) serum. Cold agglutinins were not demonstrable using normal cells, but papainized cells were agglutinated at 37° C. to a titre of 16. The indirect antiglobulin test was positive. No antibody specificity could be demonstrated. The patient's blood group was O Rh-positive. The results were interpreted as indicating the presence of a γG "non-specific" auto-antibody.

Course and Treatment. The patient was treated with prednisolone at a dose of 50 mg. daily and transfused with the packed cells from 3 l. of blood within the first week of admission. Subsequently, he improved and following the development of a reticulocytosis about 14 days after admission the hæmoglobin started to rise (Fig. 184). Improvement continued for a further 1 month. He then complained of the sudden onset of pain in the left side of the chest. He died the following day. A post-mortem examination showed an unsuspected empyema in the left pleura, from which group-A *Str. pyogenes* was grown, and active tuberculosis at the apex of the left lung in addition to signs of widespread lymphocytic leukæmia affecting the spleen, liver and lymph nodes.

Summary. A case of chronic lymphocytic leukæmia complicated by an auto-immune hæmolytic anæmia. The initial response to steroid therapy was good but death resulted from an overwhelming infection with *Str. pyogenes.*

Acute Leukæmia

A number of reports are now available which indicate that excessive hæmolysis plays a part in the causation of the anæmia of at least some cases of acute leukæmia. But the more obvious pointers to signs of hæmolysis, such as jaundice, hæmoglobinuria and reticulocytosis are seldom encountered and the direct antiglobulin test is rarely clearly positive.

Stats (1950) mentioned several cases of myeloblastic leukæmia in which he had been able to demonstrate increased hæmolysis by the sedimentation differential agglutination test and Brown, Elliott and Young (1951), using the Ashby method, reported a mean cell life-span of 23 days in a case of acute lymphatic leukæmia. Frumin and Kohn (1955) described a patient with acute granulocytic leukæmia whose direct antiglobulin test was strongly positive although there were no overt signs of hæmolysis. Hennemann (1956) found the test positive in one out of ten cases.

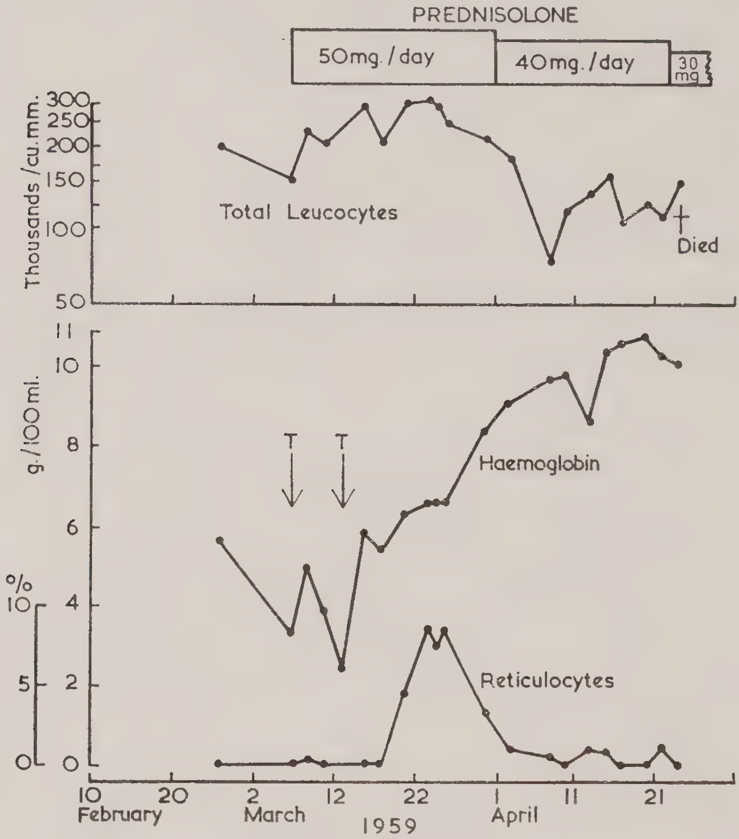

FIG. 184. Hæmatological changes in a patient suffering from chronic lymphocytic leukæmia and auto-immune hæmolytic anæmia (Case 15). Response to adrenocorticosteroid therapy. T = transfusions.

Hennemann (1956), too, referred to the sometimes difficult distinction between acquired hæmolytic anæmia and a leukæmoid reaction and genuine leukæmia with a secondary severe hæmolytic anæmia. He cited a case where at necropsy the expected signs of leukæmia were not found but instead there was abundant evidence of extramedullary erythropoiesis.

Boiron and his co-workers (1955) investigated 18 patients in all. Excluding the cases in which their studies were incomplete, they obtained evidence of increased hæmolysis in one patient using ^{14}C-2-glycine and in five out of six using the Ashby method (half-survival times of 5–40 days). They stressed that even in patients in whom their measurements indicated markedly reduced erythrocyte survival the classical signs of excessive hæmolysis were often absent.

Radioactive chromium has been generally used in later work. Wetherley-Mein, Epstein, Foster and Grimes (1958) demonstrated a clear increase in hæmolysis in four cases. Nathan and Berlin (1959b), using ^{51}Cr and ^{14}C-2-glycine, studied six patients: erythrocyte life-span was normal in one, shortened but finite in four patients, and shortened due to random destruction in one. Whitelow, Evelyn, Solvonuk and Tang (1959) studied 13 patients and found a moderate or marked reduction in the life-span of the patients' own cells in two-thirds of them. Again, they mentioned the lack of correlation between the survival data and the results of other laboratory studies; and they stressed the need for measuring the blood volume at the beginning and end of the experiments. Tudhope (1959) obtained evidence for a reduced erythrocyte life-span in four out of six patients and Bowie, Kiely, Tauxe and Stickney (1962) in seven out of eight patients; in three the survival data best fitted a semilogarithmic plot indicating random hæmolysis.

Erythræmic myelosis (Di Guglielmo's disease). There seems little doubt that, as in more typical acute leukæmia, increased hæmolysis may be a significant secondary factor in the causation of the anæmia of erythræmic myelosis. Perlick (1951–3) referred to the earlier literature and described a patient in whom hæmolysis appeared to be an important feature. The early literature and the role of hæmolysis were also reviewed by Schwartz and Critchlow (1952).

Verloop (1955) referred to four patients: the serum bilirubin was raised in all of them and the excretion of fæcal urobilinogen was markedly increased. Baldini, Fudenberg, Fukutake and Dameshek (1959) reported on 11 patients. They, too, stressed the absence of signs of overt hæmolysis. However, although the serum-bilirubin level was raised in only three patients, the fæcal-urobilinogen excretion and hæmolytic index were raised in all. ^{51}Cr studies were carried out in eight patients: the ^{51}Cr $T\frac{1}{2}$ was 8–22 days, corresponding with an erythrocyte destruction rate of 2·4–8·6 times the normal. This they considered was mainly due to the erythrocytes being intrinsically defective. In five out of six patients the urobilinogen output was much higher than that which could be accounted for by a shortened erythrocyte life-span, data which are in accord with the generally accepted concept that ineffective erythropoiesis* is a major factor in the pathogenesis of the anæmia in these patients.

* In this context the term "ineffective erythropoiesis" is meant to signify intramedullary death of developing erythroblasts and reticulocytes.

A further single case was studied by Maurice and Siegenthaler (1961). This patient's illness ran a subacute course and the ^{51}Cr T$\frac{1}{2}$ was 14 days.

Serological Findings in Acute Leukæmia

Positive direct antiglobulin tests have seldom been described in patients with acute (or subacute) leukæmia. However, amongst the few positive reports may be quoted those of Frumin and Kohn (1955) in a patient with typical acute granulocytic leukæmia and Bile, de Biasi and Ventruto (1960) in a patient in whom chloromatous tumours were found at necropsy.

A patient with subacute myelomonocytic leukæmia and hæmolytic anæmia (Case 16) is described below. This is one of the only three cases of leukæmia (other than of the chronic lymphocytic type) personally observed by the author in which the direct antiglobulin test was clearly and unmistakably positive.

Case Report: Myeloid (Myelo-Monocytic) Leukæmia; Rapid Hæmolysis of Transfused Normal Blood; Splenectomy

Case 16. The patient, H. C., a male aged 74, was first found to be anæmic and to have an enlarged liver and spleen in July 1959. He was admitted into Hammersmith Hospital in April 1956 under the care of Dr. (now Professor) Sheila Sherlock, giving a history of having received 38 transfusions in the previous 9 months.

Physical Examination. He was seen to be a frail, and markedly pale, elderly man. The liver and spleen were palpable but superficial lymph nodes did not seem to be enlarged.

Laboratory Findings. On admission, the erythrocyte count was 1·8 millions per cu. mm., hæmoglobin 5·5 g. per 100 ml., PCV 17% and reticulocyte count 0·9%. The leucocyte count was 2,200 per cu. mm., neutrophils 26%, eosinophils 1%, lymphocytes 28%, monocytes 9% and abnormal monocytoid cells 36%. The platelet count was 180,000 per cu. mm. Blood films showed only a small amount of erythrocyte anisocytosis and little or no polychromasia—most of the cells present were doubtless transfused normal cells.

Bone-marrow aspiration showed an active marrow. Erythropoiesis was relatively inconspicuous and there was an excess of promyelocytes, myelocytes and monocytoid cells; only 4% of blast cells were present. Few megakaryocytes were seen. Subsequently, an iliac-crest marrow biopsy was carried out. Sections showed that the marrow was unusually cellular and that fat spaces had almost completely disappeared. Leucopoietic cells from reticulum cells to myelocytes were particularly prominent but there were a few foci of pyknotic normoblasts. Silver staining showed a moderate increase in reticulin with some disorganization of pattern. A "myeloid" type of leukæmia was diagnosed.

The serum bilirubin was 0·6 mg. per 100 ml.; serum albumin 3·7 g. and serum globulins 4·3 g. per 100 ml. The blood urea was 48 mg. per 100 ml.

Serology. The direct antiglobulin test was positive (+) and the reaction appeared to be of the non-γ type. No abnormal antibodies could be demonstrated in the patient's serum. It was not felt that the findings necessarily indicated that antibody-induced hæmolysis was occurring.

Course and Treatment. Because of the history of needing repeated transfusions the survival of compatible normal cells was studied after labelling them with ^{51}Cr. (The presence of much transfused blood precluded the possibility of studying the patient's own cells.) The mean life-span of the labelled cells was greatly reduced (6 days) and there was a rapid and abnormal increase in radioactivity in the splenic area (Hughes Jones and Szur, 1957; Case 13, their Fig. 4).

In view of the above data, indicating that the spleen was an important site of destruction of (at least) transfused normal blood, and because of the clinical necessity for repeated transfusions, splenectomy was undertaken on the 5th June 1956. The spleen weighed 600 g.; sections showed the pulp to be packed with erythrocytes and immature and mature leucocytes. Erythrophagocytosis was conspicuous and iron was present in large amounts.

After operation, the patient developed a severe generalized arthritis, of unknown origin, which responded dramatically to prednisolone. The hæmoglobin, too, stabilized at about 8·5 g. per 100 ml. and transfusions were for a time no longer necessary. Six weeks after splenectomy, soon after discharge from hospital, he was readmitted with pyrexia and signs of pulmonary infection. The latter progressed despite vigorous antibiotic therapy and he died on 11th September 1956.

Following splenectomy, the peripheral leucocyte count rose transitorily from leucopenic levels to 23,000 per cu. mm. and then subsided to 3,000–4,000 cells per cu. mm. With the onset of the pulmonary infection a leucocytosis developed which eventually reached 100,000 cells per cu. mm.; 50–60% of the cells were polymorphonuclear neutrophils and 16–32% abnormal monocytes. The last count included 9% of blasts.

A post-mortem examination demonstrated the presence of myeloid leukæmia, multiple pulmonary abscesses and a right pleural empyema.

Summary. A case of myeloid (myelo-monocytic) leukæmia complicated by excess hæmolysis. Transient improvement followed splenectomy; pulmonary infection led to death 3 months later.

RETICULOSARCOMA AND LYMPHOSARCOMA, ETC.

Hæmolytic anæmia is a well known complication of reticulosarcoma and of malignant lymphomas other than chronic lymphocytic leukæmia, e.g., lymphosarcoma and giant follicle lymphoma (Brill-Symmers's disease). Amongst early references may be quoted the report by Singer and Dameshek (1941) of a patient who developed a severe hæmolytic anæmia associated with a lemon-size lymphosarcoma of the pancreas, and that of Aubert and Brendemoen (1949) who described a patient whose hæmolytic anæmia accompanied an abdominal tumour which at necropsy was found to be a lymphoblastoma. A little later Hagen and Watson (1951) described a patient whose hæmolytic anæmia accompanied a "reticulo-endotheliosis" and Evans and Doan (1951) referred to seven patients with giant follicle lymphoma and hæmolytic anæmia. Krah's (1951) patient suffered from reticulosarcoma.

Other relatively early reports include those of Dustin and Drochmans (1952) on a patient with lymphosarcoma, Paraf and Dausset (1952), who described a patient suffering from reticulosarcoma and hæmolytic anæmia and referred to eight similar occurrences, and Willcox (1952) on patients with reticulosarcoma and lymphosarcoma, respectively. Not infrequently, as in Hodgkin's disease, hæmolytic anæmia has developed before the appearance of overt signs of lymph-node enlargement or malignant tumour. This was true, for instance, of the cases reported by Paraf and Dausset (1952), Dustin and Drochmans (1952), Dacie (1954, p. 337, Case 21; Fig. 185) and Wasserman and his co-workers (1955).

More recent papers describing other features of unusual interest include those of Rappaport and Johnson (1955), Hennemann and Kunz (1955), Giraud, Cazal and co-workers (1955), Widmann (1957), Boulet and his co-workers (1960), Tiagi, Navani and Jain (1962), Norcross (1963) and Becker and Eddy (1964).

Rappaport and Johnson described three cases of malignant lymphoma, with auto-immune hæmolytic anæmia, in which Russell-like bodies were found in lymphocytes. Giraud and his colleagues observed the development of malignant lymph nodes (reticulosarcoma) 3½ years after the onset of paroxysmal cold hæmoglobinuria thought to be syphilitic in origin. Widmann's patient had a peripheral-blood picture suggestive of a plasma-cell leukæmia but this was associated with an underlying reticulosarcoma. The patient of Boulet and his colleagues suffered from intermittent anæmia, fever and lymph-node enlargement for 2 years before the diagnosis of reticulosarcoma could be established; while Tiagi, Navani and Jain's and Becker and Eddy's reports are illustrative of the patient who dies of an acquired hæmolytic anæmia considered to be "idiopathic" in life but shown to be secondary to lymphosarcoma *post mortem*.

Serological Findings. Most of the patients described by the authors listed in the previous paragraphs have had erythrocytes giving positive direct antiglobulin tests. This was so in the cases described by Paraf and Dausset (1952), Dacie (1954, Case 21), Rappaport and Johnson (1955), Hennemann and Kunz (1955) and Widmann (1957). In the majority of instances, but not in all, the auto-antibody has been of the cold type, e.g., in the patients described by Aubert and Brendemoen (1949), Krah (1951), Giraud, Cazal and co-workers (1955) and Tiagi, Navani and Jain (1962). An abnormally high γ-globulin level (not a macroglobulin) and auto-agglutination persisting at 37°C. were demonstrated by Hennemann and Kunz (1955) in one case.

The author's own data obtained in 11 cases are summarized in Table 64. This shows a wide variety of antibody patterns. In five cases the auto-antibody was of the warm type and in five it was of the cold type, while in one patient both warm- and cold-type antibodies appeared to be present. Three of the warm antibodies had Rh specificity and two of the cold antibodies appeared to be

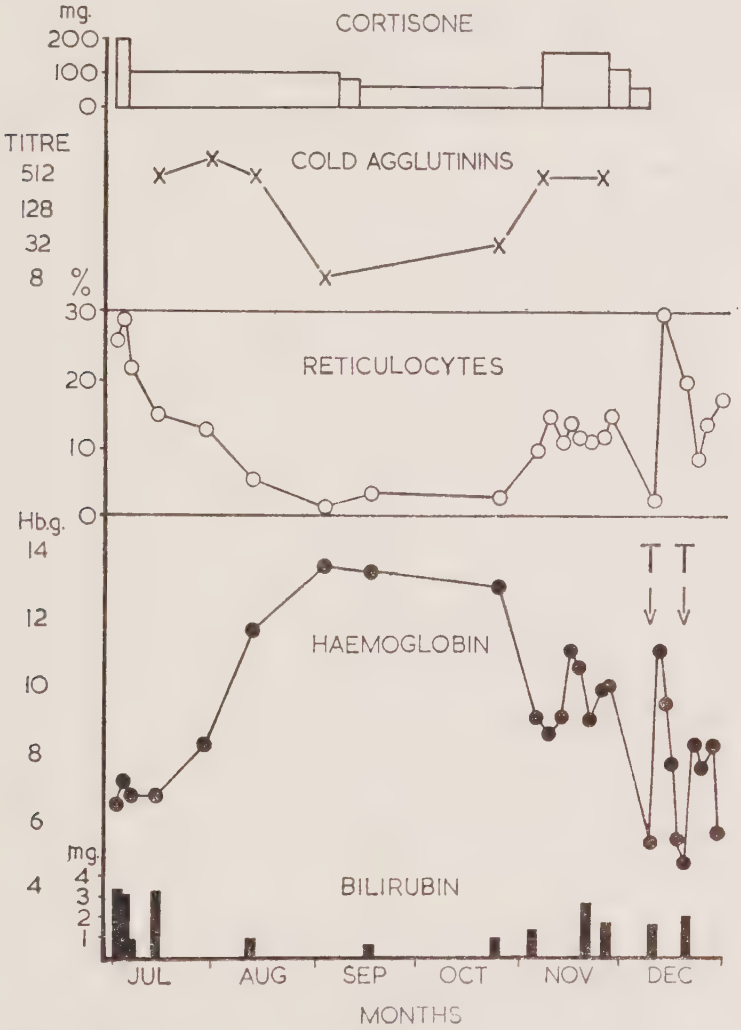

FIG. 185. Hæmatological changes in a patient suffering from auto-immune hæmolytic anæmia of the cold-antibody type and reticulosarcoma (Case 21 of Dacie (1954)). The underlying tumour did not become obvious until 2 months before her death; this was at least 1 year after the development of anæmia. The chart shows an initial good response to cortisone given in relatively small dosage. T = blood transfusions.

Table

Serological Findings in Auto-Immune Hœmolytic Anœmia Associated
(unpublished data of

Initials of patient	Sex Age	Histological diagnosis	Antiglobulin test		
			Direct		Indirect 37° C.
			Anti-γG	Anti-non-γ	
L.R.	F.58	Malignant lymphoma	+ + +	...	+ + +
L.H.	F.70	Malignant lymphoma of poorly differentiated lymphocytic type†	–	+ +	+ (pH 6·5)
A.H. (Case 19)	F.54	Nodular malignant lymphoma (follicular lymphoma) of well differentiated lymphocytic type†	+	+	...
D.M.	F.55	Monocytic leukæmia	+ + +	–	+
S.B.	F.69	Malignant lymphoma, of anaplastic reticulum-cell (or stem-cell) type†	...	+ +	–
E.D.	F.48	Tumour transitional between lymphosarcoma and reticulosarcoma	...	+	±
A.T. (Case 18)	F.68	Anaplastic reticulosarcoma†	–	+ + +	–
M.A.	F.58	Subacute myeloblastic leukæmia	–	+ +	–
J.C.-D.	M.64	Plasma-cell tumour involving spleen and lymph nodes†	–	+ + +	+
L.D.	F.51	Malignant lymphoma of poorly differentiated lymphocytic type†	+ +	+ + +	+ + +
J.H. (Case 17)	M.25	Malignant lymphoma; at first mixed lymphocytic and reticulum-cell, later pure reticulum-cell type†	+	+ +	–
D.	M.	Lymphosarcoma	+ + +	+ +	+ +
B.B.	M.56	Lymphoma, ?type (abnormal lymphoid cells in blood and skin infiltration)	–	+ + +	±
W.P.	M.74	Lymphoma	–	+ +	–
M.M.	F.58	Hodgkin's disease†	+ + +	–	–
C.E. (Case 14)	F.43	Hodgkin's disease† at biopsy; pleomorphic reticulosarcoma at necropsy†	+ + +	+ + +	–

* These sera also contained cold agglutinins (at normal titres) which were
† Histological diagnosis of Professor C. V. Harrison.
+ + + + denotes very strong naked-eye agglutination or hæmolysis;
– denotes no agglutination or hæmolysis; ... denotes no observation.

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with Malignant Reticuloses, Lymphosarcoma and Reticulosarcoma, etc. Worlledge and Dacie)

Agglutination			Lysis				Type of auto-antibody	Specificity of auto-antibody
Normal cells (titre)		Papainized normal cells (titre)	PNH cells 37° C.	Papainized PNH cells 37° C.	Papainized normal cells			
2–4° C.	20° C.	37° C.			Stored 37° C.	Fresh 37° C.		
...	< 1	16	+	+ + +	+ +	+	Warm	Anti-c + "NS"
512	64	8	+	...	...	+	Cold	"NS"
4,096	< 1	64	–	–	–	–	Warm and cold	Anti-C + anti-c (–D– cells not agglutinated)
...	< 1	1	–	+ +	±	–	Warm	"NS"
1,024	128	64 (stored cells)	–	+ +	+ +	–	Cold	"NS"
4,096	128	< 1	+ + +	+ + + +	+ + + +	+ + +	Cold	Anti-I
64,000	1,024	< 1	–	+	–	–	Cold	Anti-I
16	4	< 1	+ +	+ +	+ +	–	Cold	Anti-I (low titre)
16	4	256	–	+	–	–	Cold	Anti-I
16	< 2	256	–	+ + +	+ + +	±	Warm	"NS"
64	1	256	–	–	–	–	Warm*	Anti-e
4	< 1	16	–	+ + +	+	+	Warm	"NS"
16,000	4,000	1	–	+	–	–	Cold	Anti-I + anti-i
4	< 1	1	–	+ +	–	–	Warm	"NS"
<4	<4	4	...	...	–	–	Warm	"NS"
16	1	16	...	...	+	–	Warm*	"NS"

just active at 20° C.

+ + +, + +, + and ± denote lesser degrees of agglutination or hæmolysis; "NS" denotes specificity not demonstrated.

anti-I. One patient's serum appeared to contain both anti-I and anti-i (see also Table 65). In four patients the cold-agglutinin titre was 4096 or more. One of the sera in which cold antibodies were present also contained a warm-antibody component of Rh specificity. Four sera containing warm antibodies hæmolysed papainized erythrocytes (fresh or stored) at 37° C.

Some data on the specificity of the cold auto-antibodies in reticulosarcoma are given in Table 44 of this book (Part II, p. 497). In three cases the antibodies did *not* react as anti-I antibodies, as the antibodies found in "idiopathic" cases of cold-antibody hæmolytic anæmia seem almost always to do. More extensive data on patients with reticulosarcoma or lymphosarcoma are summarized in Table 65.

Table 65

Specificity of Antibodies in Cold Auto-Immune Hæmolytic Anæmia
(Unpublished Data of Worlledge, Whittingham and Dacie)

Type of disorder	Sera tested	Anti-I	"Anti-not-I"	Anti-i	"Non-specific"
"Idiopathic"	27	24	1	0	2
Associated with reticulosarcoma, lymphosarcoma etc.	8	5*	0	1†	3‡
Post-virus pneumonia	8	8	0	0	0

* Anti-i also present in one case.
† Anti-I also present.
‡ In two cases the antibody reacted preferentially against stored cells.

Histology of Associated Malignancy. As can be seen in Table 64, no single or characteristic histological appearance is found in tumours of the lympho-reticular system which are complicated by the development of auto-immune hæmolytic anæmia. The appearances range from those of relatively benign lymphocytic lymphoma or follicular lymphoma to anaplastic reticulosarcoma. As already mentioned, typical Hodgkin's disease is rarely found and the same is true of plasma-cell tumours.

Two cases of reticulosarcoma and hæmolytic anæmia of unusual interest are described below (Cases 17 and 18).

Case Report: Auto-Immune Hæmolytic Anæmia and Thrombocytopenic Purpura; Development of Reticulosarcoma

Case 17. A male, J. H., aged 25 years, was admitted into Hammersmith Hospital in October 1962. He gave the following history. He had had an attack of jaundice lasting 2 weeks when aged 5 years. When aged 11, axillary and inguinal lymph-node enlargement was discovered at a routine examination. A node excised at this time showed a normal architecture and a few oversized lymph follicles. The appearances did not suggest any form of follicular (or other) lymphoma (Prof. C. V. Harrison).

In March 1959, when aged 22, he suffered from acute anæmia and jaundice. The liver was tender but not enlarged and the spleen became palpable 2 days after the onset of the illness. The direct antiglobulin test was negative (Dr. W. Weiner).

He was presumed to have had an attack of acute hæmolytic anæmia and was treated with blood transfusion and prednisolone to which he responded well.

He kept well until August 1959. He then became anæmic once more, the hæmoglobin falling to 7·7 g. per 100 ml. The reticulocyte count was increased, but did not exceed 5%. The serum bilirubin rose to 9·0 mg. per 100 ml., but subsequently fell to 2·5 mg. Leucopenia was a striking feature, the minimum count being 1,400 cells per cu. mm., with 11% neutrophils. The direct antiglobulin test was negative and other tests for abnormal antibodies also gave negative results (Drs. F. J. Pick and W. Weiner). A lymph node was excised. The basic architecture of the node was retained. The follicles were enlarged and had reactive centres; in addition there were focal collections of histiocytes. The appearances suggested a reactive node rather than follicular lymphoma (Prof. C. V. Harrison).

In September 1962 he sustained a further severe episode of hæmolysis: the hæmoglobin fell to 4·7 g. per 100 ml. and the reticulocyte count rose to 30%. The patient was transferred in October 1962 to Hammersmith Hospital for further study, at the suggestion of Dr. F. J. Pick.

Physical Examination. The patient was pale and slightly jaundiced. His temperature was 101·5° F. The liver was palpable 2 cm. below the right costal margin and the spleen 3 cm. below the left costal margin. Lymph nodes were palpable in the axillæ and groins; they were freely movable and small, with the exception of a node 3 cm. in length in the left inguinal region. The patient said that this node had been this size for years.

Laboratory Findings (on admission). The hæmoglobin was 8·2 g. per 100 ml., reticulocytes 10·6%, leucocytes 2,100 per cu. mm., with 36% neutrophils, and platelets 25,000 per cu. mm. Blood films showed rouleaux formation, slight auto-agglutination and moderate anisocytosis and polychromasia (Fig. 187). Occasional erythroblasts were present.

Bone-marrow aspiration revealed a hyperplastic marrow: erythropoiesis was active and normoblastic; leucopoiesis was also active but segmented neutrophils and metamyelocytes were relatively reduced in numbers. Megakaryocytes were present in probably normal numbers.

The serum bilirubin was 8·5 mg. per 100 ml.; serum folate 3·6 μmg. per ml.; serum albumin 3·6 g. per 100 ml. and serum globulins 2·6 g. per

100 ml. The electrophoretic strip appeared normal, as were chemical tests for liver function. Tests for toxoplasmosis were negative.

The enlarged left inguinal lymph node was excised. Sections showed that the reticular architecture had been destroyed and was represented by a sparse network of fibrils. The normal structure of the node had been replaced by a mixture of lymphocytes and primitive reticulum cells. Mitoses were not frequent. A malignant lymphoma was thought probable (Prof. C. V. Harrison).

Serology. Slight auto-agglutination, which persisted at 37° C., was present. The direct antiglobulin test was positive, + with anti-γG-serum and ++ with anti-non-γ (anti-C′) serum. The indirect antiglobulin test was negative but antibody in the serum active at 37° C. was detected by the use of papainized cells. Cold agglutinins active against normal cells were present to a titre of 64 at 4° C. and 1 at 20° C. Papainized normal cells or PNH cells were not lysed at 37° C. Elution studies suggested anti-e specificity. The patient's blood group was A Rh-positive. Tests for leucocyte antibodies gave negative results but incomplete platelet antibodies were demonstrated by Dr. K. Goldsmith. LE cells were not found. The W.R. was negative.

It was concluded that a warm anti-erythrocyte auto-antibody was being formed which showed some Rh specificity.

Course and Treatment (Fig. 186). One week after admission the platelet count fell steeply to less than 10,000 per cu. mm. and platelets eventually became virtually absent; the patient then started to bleed from all previous venepuncture sites, even from punctures made several days previously. Prednisone, at a dose-rate of 80 mg. daily, failed to reverse the thrombocytopenia and after 7 days ACTH was given by intravenous infusion at a daily dose-rate of 80 units. Within 2 days the platelet count rose to 50,000 per cu. mm. and hæmorrhage ceased. The count subsequently reached about 100,000 per cu. mm. but then fell over the next 14 days to 60,000 per cu. mm. The ACTH was withdrawn after 1 week and the patient was maintained on a daily oral dose of 25 mg. of prednisone. The leucocyte count fell slightly during the period of thrombocytopenia, the minimum being 1,800 cells per cu. mm., with 29% neutrophils. The reticulocyte count was, however, maintained at 10–12%. Following the steroid therapy, the leucocyte count, too, rose gradually, along with the platelet count, and eventually reached 8,000–12,000 per cu. mm. with 70–95% neutrophils (Fig. 186).

In view of the falling platelet count, splenectomy was recommended and this was successfully carried out by Mr. R. H. Franklin on the 11th November 1962.

The *spleen* measured 14 × 8 × 4 cm. and weighed only 250 g.; it was dark purplish-red in colour. The cut surface showed the presence of numerous small semi-translucent whitish bodies up to 2 mm. in diameter. Sections showed that the spleen's general architecture was preserved: the red pulp and sinuses were congested with blood and the Malpighian bodies were hyperplastic. Foamy macrophages and erythrophages were scattered in the pulp and sinusoids. No changes were present to justify a diagnosis of a lymphoma. A lymph-node removed with the spleen was considered to be normal (Prof. C. V. Harrison).

Splenectomy was followed by a dramatic rise in platelet count which reached a maximum of 600,000 10 days after the operation; the leucocyte count rose to a maximum of 17,000 per cu. mm. 2 days post-operatively. The platelet count, however, fell as rapidly as it had risen and it had dropped to 1,000 per cu. mm. by the 30th November 1962, 19 days after splenectomy. The leucocyte count had fallen to 4,800 per cu. mm. by the 25th November.

Steroid therapy was reintroduced. ACTH was given at a dose-rate of 60 units daily and prednisone at a dose-rate of 45 mg. daily; the latter was subsequently increased after 3 days to 90 mg. The platelet count slowly rose to about 10,000 per cu. mm. Fluoxymesterone was given from the 13th December 1962 onwards at a daily dose-rate of 6 mg.

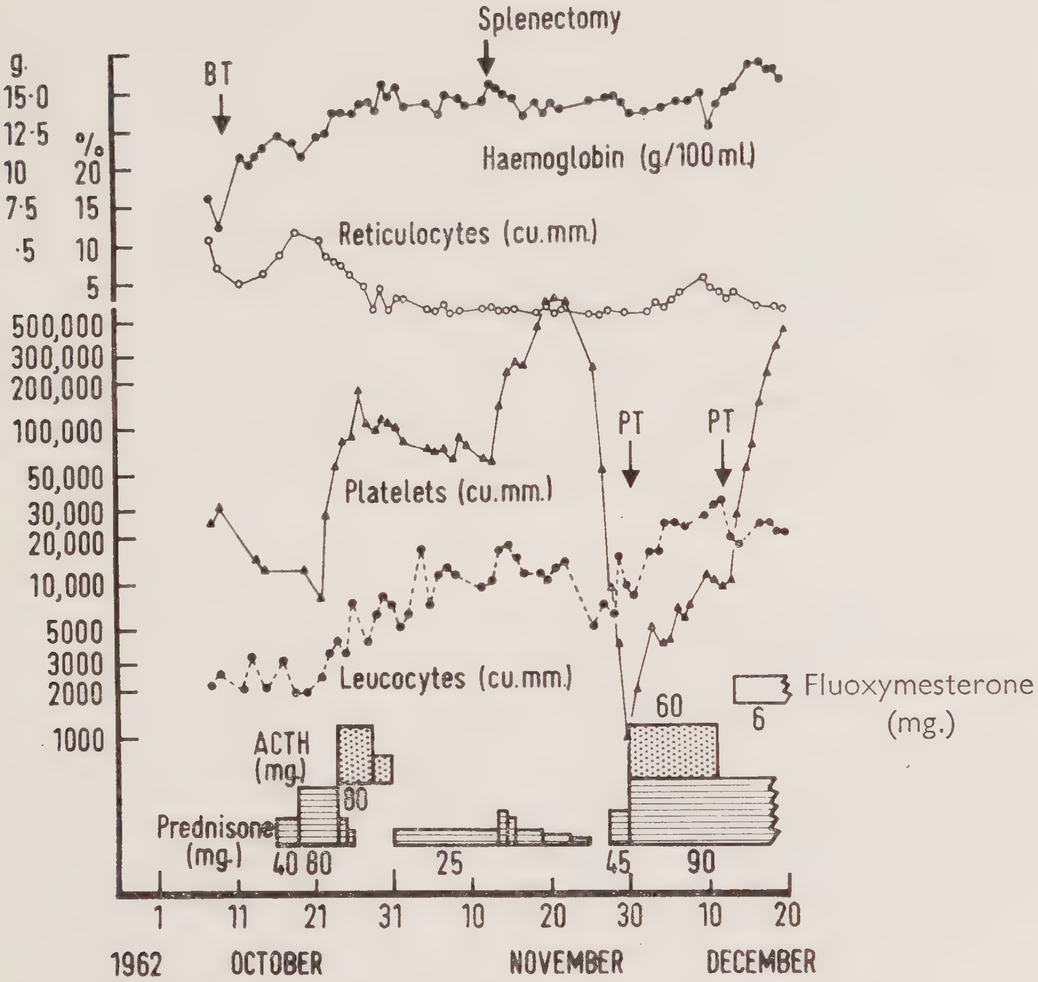

FIG. 186. Hæmatological changes in a patient suffering from auto-immune hæmolytic anæmia, thrombocytopenic purpura and leucopenia, associated with a malignant lymphoma (Case 17). The chart shows his fluctuating course and response to splenectomy and steroid therapy. BT = blood transfusion; PT = platelet transfusions.

On the following day, the platelet count was 26,000 per cu. mm. and 4 days later 200,000 per cu. mm. The leucocyte count appeared to respond promptly to the steroid therapy and reached a maximum of 32,000 per cu. mm., with 89% neutrophils on the 12th December. During all this time the hæmoglobin had been well maintained, at 13 g. per 100 ml. or more. The patient was discharged from hospital on the 20th December 1962, with 15·2 g. hæmoglobin per 100 ml., 20,000 leucocytes per cu. mm. and 400,000 platelets per cu. mm. The direct antiglobulin test had become only weakly positive (±).

Further Progress. Details of this patient's subsequent progress have kindly been provided by Dr. F. J. Pick. In February and April 1963 he appeared to be well: the hæmoglobin was normal and there was a slight to moderate leucocytosis and thrombocytosis. In August 1963, however, he felt unwell and had a purpuric rash. The direct antiglobulin test was then strongly positive and the serum contained a large amount of anti-D auto-antibody (Dr. W. Weiner). By May 1964 the abnormal antibodies had disappeared. In September 1964 enlarged nodes were found to have developed in the inguinal region and a tumour was palpable in the right hypochondrium. Biopsy of a node showed a malignant lymphoma, of reticulum-cell type. The architecture of the node was lost and the normal structure had been replaced by sheets of large cells with large vesicular nuclei which had fairly dense chromatin and prominent nucleoli. Polymorphism and mitotic figures were frequent. Reticulin fibrils formed an intimate meshwork amongst the cells (Prof. C. V. Harrison).

Conclusion and Summary. The patient has been suffering from an auto-immune hæmolytic anæmia and possibly, too, from auto-immune granulocytopenia and thrombocytopenia. The clinical course was characterized by a series of episodes of hæmolysis or purpura, and the variability of the serological findings was similarly remarkable. A noteworthy feature has been the slow evolution of a lymphoma over a number of years, eventually terminating in overt reticulosarcoma.

Case Report: Auto-Immune Hæmolytic Anæmia of the Cold-Antibody Type Associated with Reticulosarcoma

Case 18. A female, A. T., aged 68, was admitted to hospital in May 1959 because of bleeding from the gums. She also complained of pain in both hips and ankles. She was referred to Hammersmith Hospital as a possible case of myelomatosis in June 1959.

Physical Examination. She was seen to be pale but she was not clinically jaundiced. The liver was palpable 10 cm. below the right costal margin but the spleen was not felt. Several enlarged lymph nodes were palpable in the right axilla but not elsewhere.

Radiographs showed some degenerative arthritic changes in the cervical spine and spondylosis and disc degeneration in the dorsal spine. There was no X-ray evidence of myelomatosis.

Laboratory Investigations. On admission, the hæmoglobin was 8·5 g. per 100 ml., reticulocytes 4·8%, leucocytes 8,000 per cu. mm., with 49% neutrophils, 1% myelocytes, 34% lymphocytes, 2% monocytes and 14% abnormal cells. Morphologically, most of the abnormal cells looked intermediate in type between plasma cells and lymphocytes. The nuclear chromatin was more open and variegated than in typical lymphocytes, but the nucleus itself was more often than not central or only slightly eccentric in position. The cell cytoplasm showed varying degrees of basophilia. A minority of the cells appeared to be immature or partly mature plasmacytes (Fig. 188). The platelet count was 156,000 per cu. mm. Blood films showed marked cold auto-agglutination, some anisocytosis and polychromasia and the abnormal cells

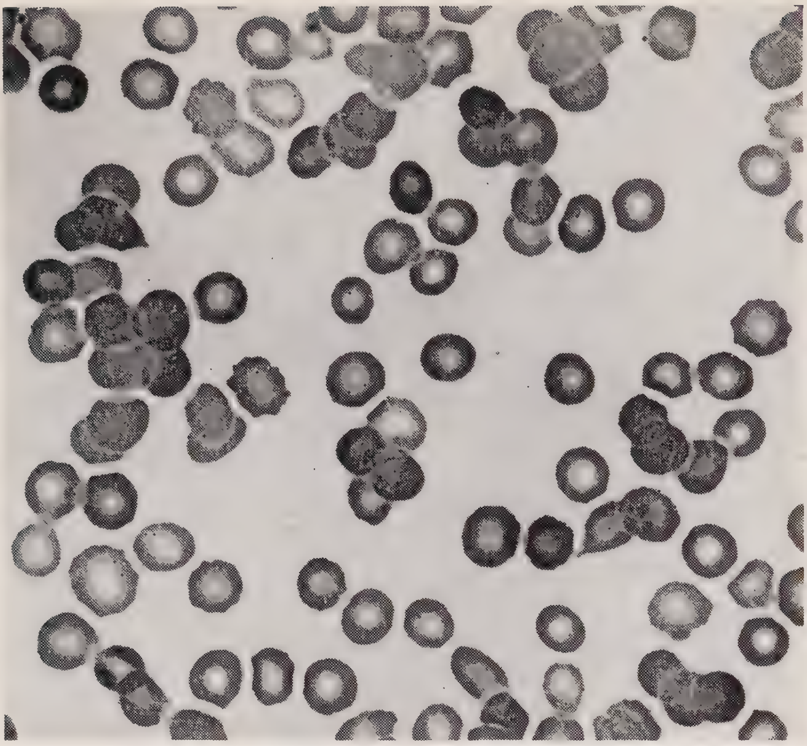

Fig. 187. Photomicrograph of a blood film from a patient suffering from auto-immune hæmolytic anæmia, thrombocytopenic purpura and leucopenia associated with a malignant lymphoma (Case 17). There is moderate anisocytosis, rouleaux formation and slight auto-agglutination. × 700.

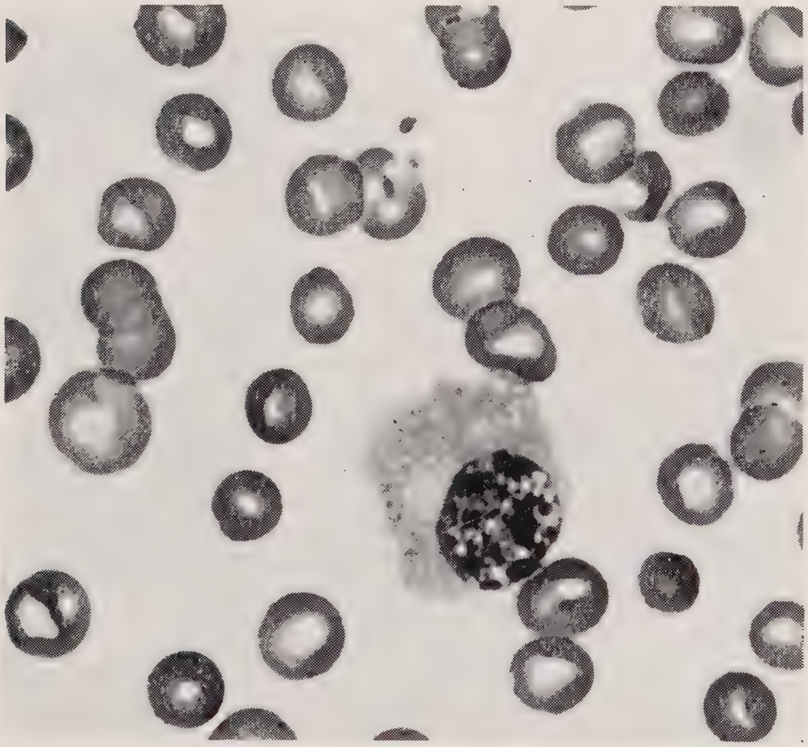

Fig. 188. Photomicrograph of a blood film from a patient suffering from auto-immune hæmolytic anæmia of the cold-antibody type associated with an underlying malignant lymphoma (Case 18). The leucocyte photographed is thought to be a partly mature plasmacyte. × 1000.

To face p. 748.

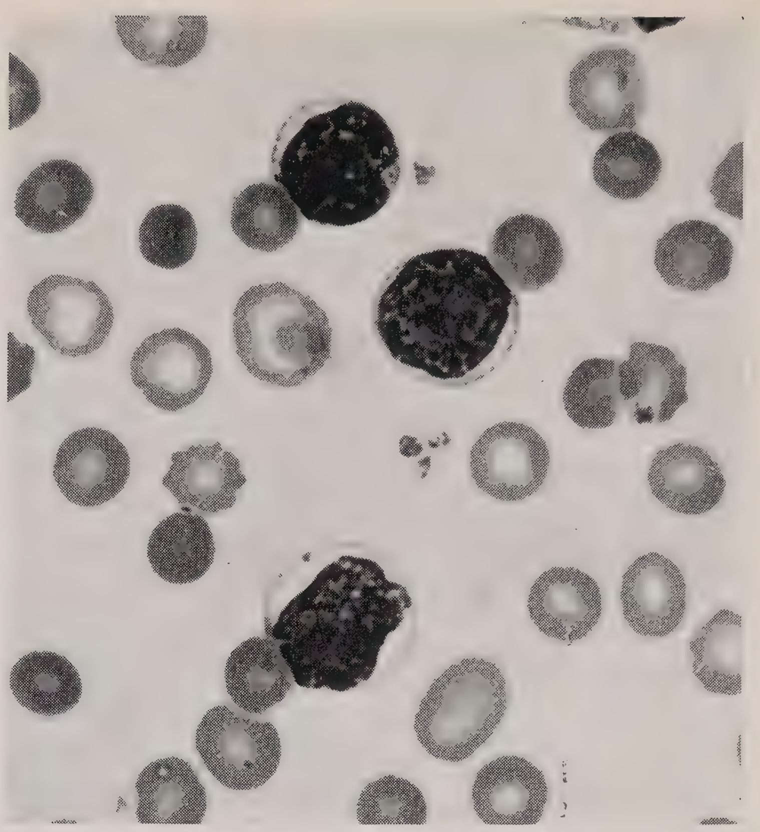

FIG. 189. Photomicrograph of a blood film from a patient suffering from auto-immune hæmolytic anæmia of the cold-antibody type associated with an underlying malignant lymphoma (Case 18). The leucocytes present resemble partly mature lymphocytes. Some microspherocytes are also present. ×1000.

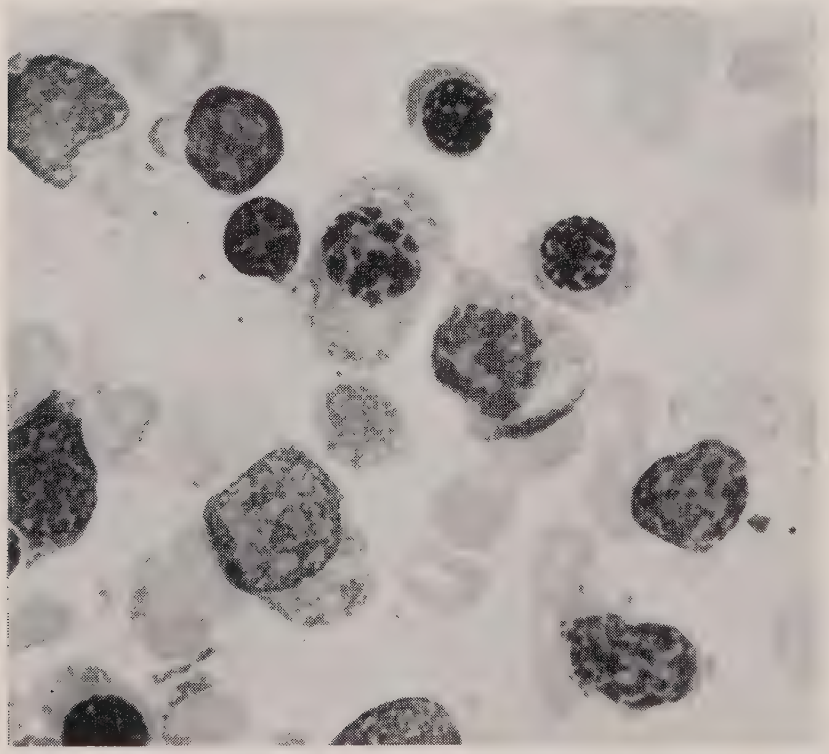

FIG. 190. Photomicrograph of bone-marrow cells from a patient with auto-immune hæmolytic anæmia of the cold-antibody type and an underlying malignant lymphoma (Case 18). Cells are present which range in morphology between plasmacytes and lymphocytes. ×1000.

mentioned above. Films made on slides warmed to 37° C. showed marked rouleaux formation and little or no auto-agglutination.

The erythrocyte osmotic fragility was increased: lysis commenced in 0·60% NaCl and the MCF was 0·475% NaCl. The patient's serum contained methæmalbumin and free hæmoglobin; haptoglobins were absent. The urine was normal.

After X-ray therapy the patient became more anæmic: the hæmoglobin fell to 6·3 g. per 100 ml., leucocytes to 1,900 per cu. mm. and platelets to 30,000 per cu. mm. The reticulocyte count rose, 14.3% being the maximum recorded. Plasma cells (up to 5%) were constantly present in peripheral blood films.

Bone-marrow puncture yielded a hyperplastic marrow in which erythropoiesis, normoblastic in type, was conspicuous. In addition, however, many cells were present which in appearance ranged between large lymphocytes and typical mature plasma cells (Fig. 190). A tentative diagnosis of myelomatosis was made, which appeared to be confirmed by the serum-protein findings (see below).

The *serum proteins* totalled 8·9 g. per 100 ml., albumin 2·9 g. and globulins 6·0 g. There was a very marked and sharp peak in the γ-globulin region. The ESR was 152 mm. in 1 hour.

Clinical Progress. The patient received X-ray therapy to the lumbosacral region, being given 3,000 r. in the course of 18 days. She subsequently became more anæmic (see above) and was transfused with the packed cells derived from 2 l. of blood. Six days later she was noticed to be jaundiced and the serum bilirubin was found to be 3·5 mg. per 100 ml.

Following the X-ray therapy she was given prednisolone at an initial dose-rate of 40 mg. a day. She was discharged from hospital on the 21st August 1959 at a time when the hæmoglobin had risen to 9·6 g. per 100 ml. Prednisolone was continued at a dose-rate of 30 mg. a day. This was reduced to 15 mg. a day in January 1960.

A serum sample from October 1959 has been re-studied by Dr. J. R. Hobbs. The total protein was 9·3 g. per 100 ml., with albumin 4·5 g. per 100 ml. and globulins 4·8 g. per 100 ml., of which 3·4 g. was an M protein which produced the sharp tall peak on electrophoresis. Immuno-electrophoretic analysis showed that the patient was forming a γMK paraprotein and that this was associated with mild immune paralysis.

Serology. The direct antiglobulin test was positive (+ + +) using anti-non-γ (anti-C′) serum and negative with anti-γG serum. Cold agglutinins of anti-I specificity were present to a titre of 64,000 at 4° C. and 1,024 at 20° C. Auto-agglutination was just detectable at 31° C. but not at a higher temperature. Papainized normal cells, but not unmodified cells, were weakly agglutinated (and hæmolysed) at 37° C. The indirect antiglobulin test was weakly positive at 37° C. Normal erythrocytes were hæmolysed by the patient's acidified serum at 20° C. Serum complement activity was reduced (28 units, control 78 units).

Further Progress. The patient was admitted for further X-ray therapy in February 1960 because of increasing pain in the hip and lumbar region. A repeat bone-marrow biopsy showed the presence of approximately 9% of abnormal plasma cells. Total proteins were 7·7 g. per 100 ml. of which 5·0 g. were globulins.

In February 1961, because of increasing anæmia, she was admitted into hospital for blood transfusion. Bone-marrow biopsy was again undertaken. This time only a small number of plasma cells (3·5%) were identified, while abnormal lymphoid cells were present in greater numbers (16%). Similar cells could be found in peripheral blood films. The highest leucocyte count at this time was 26,000 per cu. mm., neutrophils 16% and lymphocytes 83%. Compared with the abnormal cells in the peripheral blood at the start of the patient's illness, these cells were more like small or medium-sized lymphocytes, although the nuclear chromatin was still less homogeneous than in normal lymphocytes. Many of the cells had only limited amounts of cytoplasm (Fig. 189). Cells closely resembling plasmacytes appeared to be absent. The serum globulins had fallen to 4·4 g. per 100 ml.

In July 1961 the patient's right breast was noticed to have become swollen and firm, and drill biopsy showed the presence of a malignant lymphoma—large reticulum cells were present as well as lymphoblast-like cells in sheets. Radiographs showed deposits also in the lumbar region and right iliac crest. These areas were treated by X-ray therapy. The serum globulins had fallen further to 3·1 g. per 100 ml.

Further X-ray therapy was given in February 1962 for tumour deposits in the left forearm.

Later in February 1962 she was admitted to hospital for the last time. Lymph nodes had become palpable in the groins and axillæ and the spleen could also be felt. The blood urea rose terminally to 370 mg. per 100 ml.

A marrow puncture showed only 0·2% of abnormal plasma cells; 3% of the cells were abnormal lymphoid cells but, in addition, about 30% of the cells were represented by bare nuclei thought to be derived from lymphoid cells.

During the whole course of the patient's illness the cold agglutinin was present at high and virtually unchanged titre and persistent anæmia and reticulocytosis suggested that excess hæmolysis was continuing. The serum bilirubin ranged from 0·7 to 2·5 mg. per 100 ml. Hæmoglobinuria was, however, never noticed and hæmosiderin was not demonstrated in the urine deposit. The patient never complained of Raynaud's phenomena. The atypical "plasma cells" were not seen in peripheral blood films except at the first admission in 1959.

The patient died on the 14th March 1962. Post-mortem examination showed reticulosarcoma with main deposits in nodes in the groins and axillæ, the right ulna and soft palate; also acute pyelonephritis and cystitis.

Conclusion and Summary. The patient presented with signs of an auto-immune hæmolytic anæmia of the cold-antibody (anti-I) type and abnormal plasma cells in the marrow and peripheral blood which suggested a diagnosis of myelomatosis. These cells subsequently largely disappeared and widespread lesions developed which biopsy and necropsy showed were due to reticulosarcoma.

Macroglobulinæmia

It is becoming more and more clear that Waldenström's syndrome of macroglobulinæmia often has as its basis an underlying malignant lymphoma. While it is unusual for such patients to suffer also from an overt hæmolytic anæmia the association is not really rare.

Schubothe (1953) referred briefly to a patient with severe auto-immune hæmolytic anæmia who also had manifest macroglobulinæmia. Fankhauser, Arnold, Schaub and Lapp (1956) reported on two patients: one had an auto-immune hæmolytic anæmia for 1 year before macroglobulinæmia was discovered; the other had lymph-node enlargement (Mikulicz's syndrome) with macroglobulinæmia, a severe auto-immune hæmolytic anæmia developing subsequently. A further possible case was reported by Bousser and Boivin (1957).

Kappeler, Krebs and Riva (1958) in an extensive review referred to previously published cases of macroglobulinæmia and to 21 patients of their own. None of the latter, however, developed overt hæmolytic anæmia, but some signs of probable hæmolysis (increased reticulocyte count, diminished osmotic resistance, bilirubinæmia and positive direct antiglobulin test) were found in several of them.

A further patient presenting clear signs of hæmolysis was reported by van Gils and Loonstra (1959) and two patients were described by Imhof, Baars and Verloop (1959), who also referred to six additional cases previously described in the literature. Aavseth, Ofstad and Torvik (1961) described a patient with an illness of 13 years' duration in which hæmolytic anæmia was conspicuous in the later stages; at necropsy there was widespread lymph-node enlargement due to lymphosarcoma. Finally, Cline, Solomon, Berlin and Fahey (1963) described erythrocyte life-span measurements carried out on 10 patients, using ^{14}C-glycine and DF^{32}P as well as ^{51}Cr. In eight patients the life-span was shortened although not very markedly (the minimum ^{51}Cr $T\frac{1}{2}$ was 22 days). One patient had a positive antiglobulin test and in another (with a negative antiglobulin test) radioactivity accumulated in the spleen suggesting that the organ was a site of hæmolysis.

Serological Findings

These have seldom been described in detail. However, the direct antiglobulin test has often been recorded as positive, as in the patients described by Fankhauser and his co-workers (1956 (2 cases)), Bousser and Boivin (1957), Kapeller, Krebs and Riva (1958 (3 cases)), van Gils and Loonstra (1959), Aavseth, Ofstad and Torvik (1961) and Cline and his co-workers (1963 (1 case)). The serum of van Gils and Loonstra's patient also contained a raised titre of cold agglutinins.

The case history of a patient with lymphosarcoma, macroglobulinæmia and hæmolytic anæmia studied by the author is described below (Case 19).

Case Report: Lymphosarcoma with Macroglobulinæmia and Auto-Immune Hæmolytic Anæmia

Case 19. A woman, A. H., aged 52, was referred to hospital in May 1952 with many large discrete lymph nodes in both axillæ. Neither spleen nor liver was palpable. There was some œdema of the ankles.

She was mildly anæmic, the hæmoglobin being 11·8 g. per 100 ml., and serum-protein estimation showed a reversed albumin:globulin ratio, with albumin 2·8 g. per 100 ml. and globulins 4·5 g. per 100 ml. Lymph-node biopsy was carried out and a diagnosis of lymphoid follicular reticulosis was made. She was treated by X-ray therapy and the enlarged nodes disappeared. Next, she was admitted into hospital in August 1952 because of bilateral pleural effusions and œdema of the legs and ankles. She also had proteinuria. She improved on chest aspiration and salt restriction.

In March 1954 she was admitted into Hammersmith Hospital following an upper respiratory tract infection. She was found to be pale and slightly jaundiced and lymph nodes in the supraclavicular regions, axillæ and groins were easily palpable. The spleen and liver were also just palpable. There was œdema of the ankles. She was diagnosed as suffering from a lymphoma, the nephrotic syndrome and acquired hæmolytic anæmia.

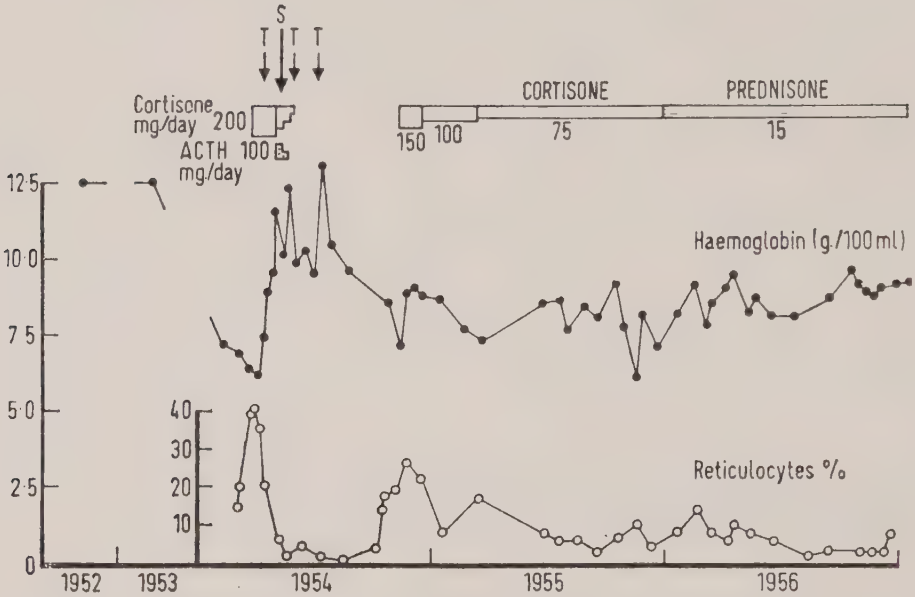

FIG. 191. Hæmatological data in a patient suffering from auto-immune hæmolytic anæmia, macroglobulinæmia and an underlying lymphosarcoma (Case 19). There was some response to steroid therapy but splenectomy resulted in little or no benefit. T = transfusions; S = splenectomy.

Laboratory Findings. On admission, the hæmoglobin was 6·8 g. per 100 ml., erythrocyte count 1,700,000 per cu. mm., PCV 19·5% and reticulocyte count 16%. The leucocyte count was 7,000 per cu. mm., with 64% neutrophils, and platelet count 370,000 per cu. mm. Blood films showed some rouleaux formation, moderate anisocytosis and marked spherocytosis and polychromasia (Fig. 192).

The serum bilirubin was 1·3 mg. per 100 ml. The total serum protein was 7·5 g. per 100 ml., 3·4 g. albumin and 4·1 g. globulins, and electrophoresis showed a high and narrow peak in the γ-globulin region.

Bone-marrow aspiration showed active erythropoiesis but no signs of a lymphoma.

Serology. The direct antiglobulin test was positive, and the reaction

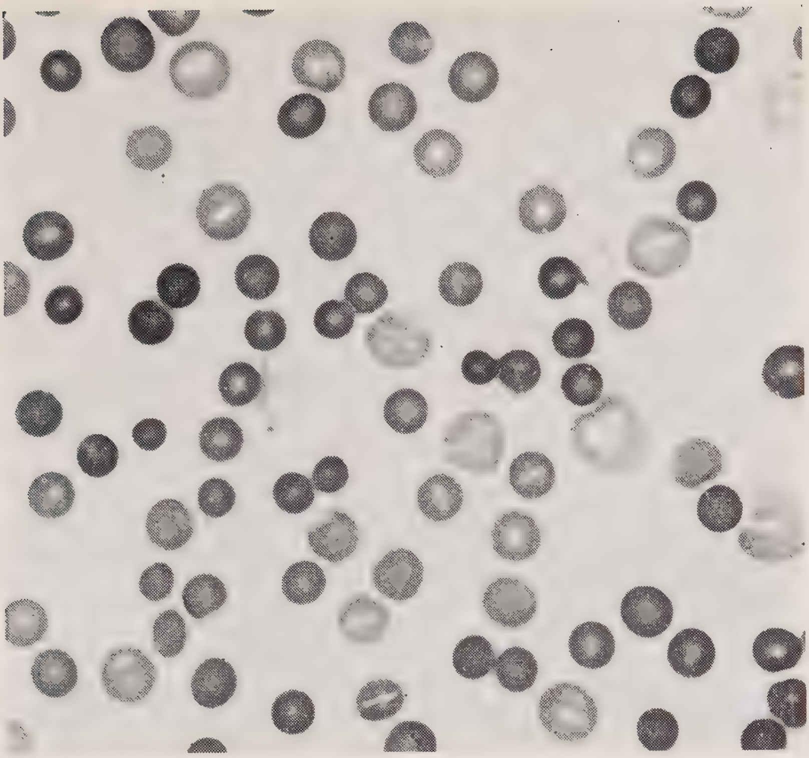

FIG. 192. Photomicrograph of a blood film of a patient suffering from auto-immune hæmolytic anæmia, macroglobulinæmia and an underlying lymphosarcoma (Case 19). Microspherocytosis, anisocytosis and anisochromasia are conspicuous. Some of the spherocytes are adhering together in pairs. ×700.

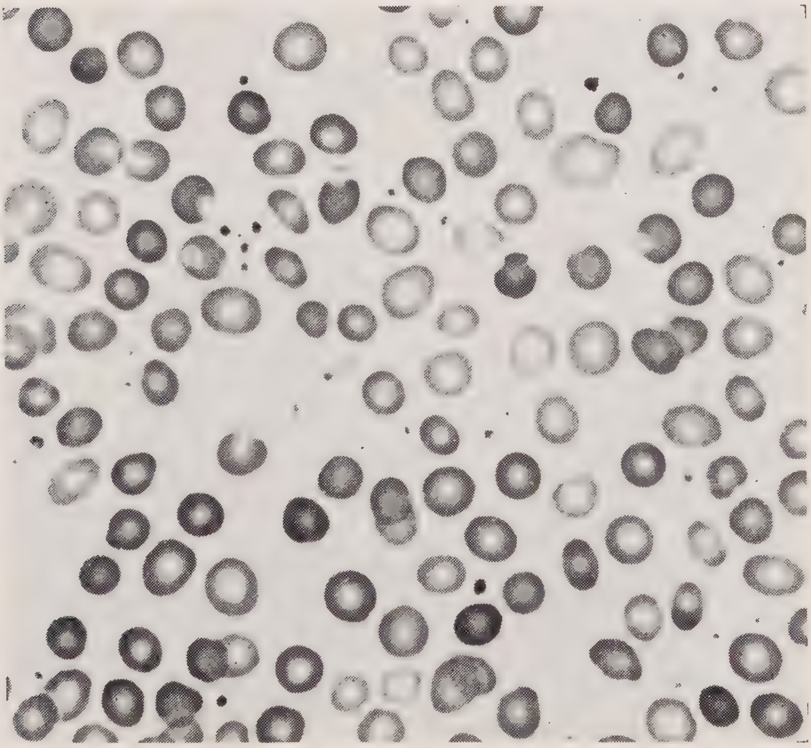

FIG. 193. Photomicrograph of the blood film of a patient suffering from myelosclerosis and excessive hæmolysis (Case 20). There is moderate anisocytosis, anisochromasia and microspherocytosis; there is also a tendency to ovalocytosis and some of the cells show a "pincered" effect. ×700.

To face p. 752.

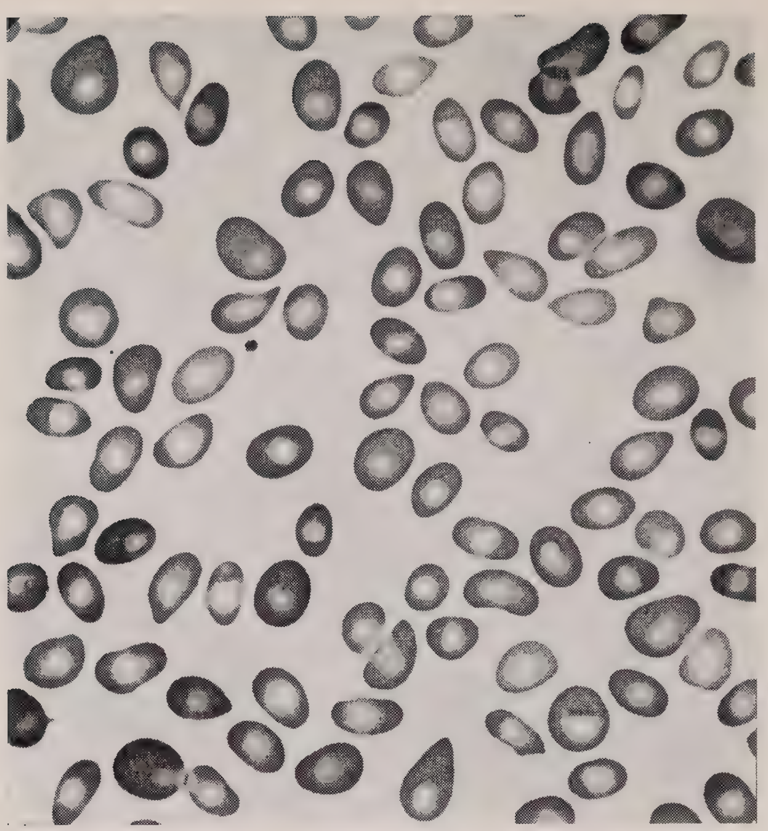

Fig. 194. Photomicrograph of a blood film of a patient suffering from myelosclerosis (Case 21). Before splenectomy. Anisocytosis, poikilocytosis and macrocytosis are conspicuous. ×700.

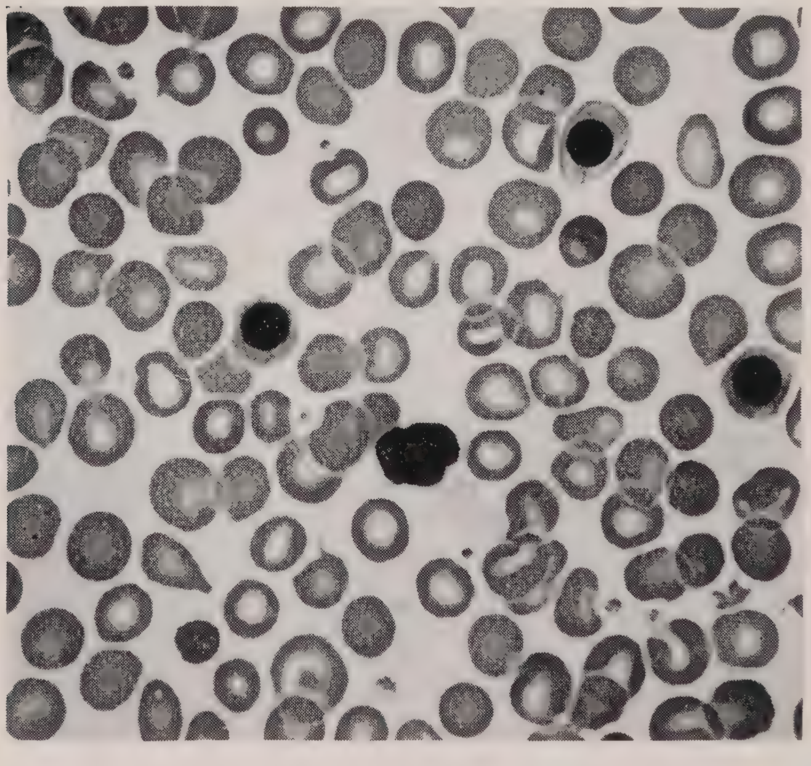

Fig. 195. Photomicrograph of a blood film of a patient suffering from myelosclerosis (Case 21). After splenectomy. Many normoblasts are present and the erythrocytes now show less variation in shape. Pappenheimer bodies and target cells are present. ×700.

was partially inhibited by γ globulin. Eluates prepared from the patient's erythrocytes appeared to react preferentially with e-positive cells; *-D-/-D-* cells were not agglutinated. Cold agglutinins were also present: normal cells were agglutinated by the patient's serum to a titre of 4,096 at 4° C. and to a titre of 1 at 20° C. Neither papainized normal cells nor PNH cells were hæmolysed at 37° C. It was concluded that the patient was forming an anti-erythrocyte auto-antibody of the warm type, of probable Rh specificity, and also a cold agglutinin, the activity of which just reached 20° C.

Further Studies, Course and Progress. The patient was treated with cortisone at a dose rate of 200 mg. per day. Some improvement resulted and the hæmoglobin rose to 11·8 g. per 100 ml. (Fig. 191). The life-span of the patient's erythrocytes studied by Dr. (now Professor) P. L. Mollison by the ^{51}Cr method before and during steroid therapy was 8 days and 17 days, respectively.

Splenectomy was carried out in May 1954 and for a time the hæmoglobin stabilized at about 10 g. per 100 ml. without further treatment with steroids. Convalescence, however, was retarded by an infected incision, which only partially healed leaving a sinus. By October 1954 the hæmoglobin had fallen to 7·1 g. per 100 ml. and cortisone therapy (150 mg. daily) was restarted, with a rise in hæmoglobin to 9·4 g. per 100 ml.

The spleen weighed 150 g. only. Sections showed no signs of a lymphoma. The Malpighian bodies appeared to be atrophic and the pulp was relatively bloodless. There was, however, hyperplasia of the reticulum cells of the spleen pulp and of the littoral cells of the sinuses. Erythrophagocytosis was not obvious but the spleen, nevertheless, contained a great deal of iron-containing pigment. A biopsy of the liver showed a few foci of lymphocytic infiltration. An abdominal lymph node appeared normal.

The patient's general condition gradually deteriorated. In June 1955 she experienced an attack of herpes zoster and during 1955 she was admitted into hospital four times suffering from respiratory infections. In February 1956 the abdominal sinus, which had never healed, was reopened and drained. The source of the continuing infection could not be determined but it appeared likely that she had a subphrenic abscess and fæcal fistula. No further surgery appeared, however, practicable. In September 1956 she was admitted into hospital for the last time: she had a fresh chest infection and the abdominal sinus was still discharging. Submandibular and cervical glands were palpable and she had become emaciated. She died on 22nd January 1957.

The patient's serum contained throughout her illness a large amount of a gamma globulin which on electrophoresis gave a high and narrow peak and which on ultracentrifugation was demonstrated to be a macroglobulin. The total globulin content of the serum varied between 3·9 and 5·5 g. per 100 ml. The serological findings remained substantially unchanged throughout.

Post-mortem examination showed a striking enlargement of all lymph nodes as well as of the liver, malignant infiltration of the diaphragm and the posterior abdominal wall, a subphrenic abscess communicating with the colon and abdominal wall, and ascites. Sections showed the tumour to be a well differentiated lymphosarcoma. The histology of the kidney indicated a nephrotic glomerulonephritis. Sections of the bone marrow did not show tumour cells, but in some areas large cells with small nuclei appeared to be phagocytosing globulin (? the macroglobulin).

Conclusion and Summary. The underlying basis of this patient's illness was lymphosarcoma. Auto-immune hæmolytic anæmia was, however, at one time the dominant clinical feature. Splenectomy had little effect on the blood picture and post-operative infection persisted. The patient's serum contained a large amount of macroglobulin, cold agglutinins at moderately high titre and also a warm auto-antibody which probably had Rh specificity.

MULTIPLE MYELOMA

Signs of overt hæmolytic anæmia are rarely met with in multiple myeloma and the association seems definitely to be less frequent than in macroglobulinæmia. The author has not yet personally investigated a patient with hæmolytic anæmia in whom the diagnosis of multiple myeloma was unassailable (*cf.*, Case 18). However, one patient (J. C.-D., Table 64) had a plasma-cell tumour involving the spleen and lymph nodes but apparently sparing the bone marrow. There are, nevertheless, a few accounts in the literature which suggest that hæmolysis was slightly to moderately increased in patients thought to have multiple myeloma.

Positive direct and indirect Coombs tests were reported by Hennemann, Falck and Gillert (1953) in a patient suffering from what appeared to be typical multiple myeloma. The hæmoglobin was, however, 11·3 g. per 100 ml. and 4·2% was the highest reticulocyte count recorded. Butterworth, Frommeyer and Riser (1953) described an interesting case of plasma-cell leukæmia in which phagocytosis of erythrocytes by immature plasma cells was a conspicuous finding. The patient was severely anæmic and, although no studies to prove the point had been carried out, Butterworth and his colleagues concluded that the erythrophagocytosis must have been an important factor in the pathogenesis of the anæmia.

Hennemann (1955–6) referred to one positive antiglobulin test (? the case referred to above) out of 17 patients tested. Forster and Moeschlin (1954) described a case of extramedullary plasmacytomatosis with enlargement of lymph nodes, liver and spleen, plasma cells in the peripheral blood (30% of 6,700 per cu. mm.) and auto-immune hæmolytic anæmia with a positive antiglobulin test. But only 10% of the marrow cells were plasma cells and the disorder was considered to be an example of "leukæmic plasmacytomatosis" of lympho-reticular origin rather than a variant of multiple myeloma.

Two apparently typical cases of multiple myeloma were described by Massei and Pallone (1957): although the reticulocyte counts were not high, pigment-excretion studies suggested increased hæmolysis and the direct and indirect antiglobulin tests were positive. Bohrod and Bottcher (1963) have recently described an unusual case in which widespread protein phagocytosis by reticulo-endothelial cells was a conspicuous feature; increased hæmolysis was assumed on the basis of a large transfusion requirement.

Erythrocyte life-span studies seem seldom to have been carried out in cases of multiple myeloma. However, Cline and Berlin (1962) have published the results of an extensive study based on 12 patients. In nine out of 11 patients who were anæmic evidence of a slight to moderate impairment of erythrocyte life-span was obtained using the ^{51}Cr, ^{14}C-glycine and DF^{32}P methods: the minimum ^{51}Cr T$\frac{1}{2}$ was 16 days and the minimum mean life-span estimated with DF^{32}P was 33 days. Using ^{14}C-glycine, evidence for both a random and finite shortening of life-span was obtained; but the predominant pattern was of a destruction of older cells beginning after 40–60 days. There were indications that azotæmia *per se* was a factor in bringing about the hæmolysis. Bowdler and Prankerd (1962) have recorded, too, a shortened erythrocyte life-span (15 days) in a patient considered to have a latent hæmolytic anæmia.

Serological Findings. The direct antiglobulin test is usually negative in cases of multiple myeloma, but as referred to above, positive reactions have occasionally been reported. Matthias's (1964a) recent data—five positive reactions in 21 patients—suggest that the frequency of positive tests may be greater than the previous literature has indicated.

Other Reticuloses

An increased rate of hæmolysis has been reported as a feature of several reticuloses of obscure nature.

Histiocytic Medullary Reticulosis

Four patients were described by Scott and Robb-Smith (1939): erythrophagocytosis was conspicuous in lymph nodes and spleen. The antiglobulin test was reported to be positive in a patient described by Willcox (1952).

Greenberg, Cohen, Pease and Kyle (1962) reviewed 47 cases and also described a personally studied case. They concluded that increased hæmolysis contributed to the patients' anæmia and that in a few cases it had probably been of major importance. Lynch and Alfrey (1965) have recently reported detailed studies in one case. Again, it seemed likely that increased hæmolysis as a result of erythrophagocytosis by histiocytes was an important factor; the ^{51}Cr T$\frac{1}{2}$ was 16·5 days.

Familial Hæmophagocytic Reticulosis

A family affected by this rare disorder was reported by Farquhar and Claireaux (1952) and Farquhar, MacGregor and Richmond (1958). ^{51}Cr studies showed a marked shortening of the erythrocyte life-span in one child (^{51}Cr T$\frac{1}{2}$ 5 days) and a minor degree of shortening in the case of the child's father (^{51}Cr T$\frac{1}{2}$ 20 days).

MacMahon, Bedizel and Ellis (1963) described under the title "familial erythrophagocytic lymphohistiocytosis" the occurrence in two infant siblings of a fatal disorder which they considered to be allied to but not exactly the same as familial hæmophagocytic reticulosis. Spherocytosis, erythroblasts and polychromasia were noted in the blood film of one of the infants and splenectomy was carried out with temporary improvement. Sections of the spleen showed hyperplasia of reticulum cells and much erythrophagocytosis. At necropsy, the livers of both infants were found to be infiltrated with lymphocytes and histiocytes.

MacMahon and his colleagues, in reviewing the literature, discussed the relationship between the illness of the infants they studied, Letterer-Siwe's disease and histiocytic medullary reticulosis, and concluded that the three conditions were probably distinct entities.

Varadi, Gordon and Abbott (1964) described a further case of probable familial hæmophagocytic reticulosis—although with a negative family history. More recently, Goodall, Guthrie and Buist (1965) have reported a family of three affected siblings, in which it seemed likely that excessive erythrophagocytosis was one of the causal mechanisms leading to anæmia. The direct antiglobulin test was reported as negative or not commented on in all the cases referred to above.

Letterer-Siwe's Disease

Lipton (1954) described two children who showed a hæmolytic and pancytopenic (? hypersplenic) syndrome, and referred to earlier reports in the literature where raised reticulocyte counts, erythroid hyperplasia of the marrow and erythroblastæmia were mentioned.

"*Histio-plasmocytic Reticulosis*"

Hennemann and Kunz (1955, Case I) described a patient suffering from an obscure reticulosis in whom the direct antiglobulin test was positive and cold agglutinins were present in high titre. The γ-globulin concentration was raised. Forster and Meoschlin's (1954) patient may have been suffering from the same disorder (see p. 754).

Osteopetrosis

This is another rare disease in which excessive hæmolysis has been demonstrated. Engfeldt, Karlberg and Zetterström (1955) and Zetterström (1957) referred to patients in whom there appeared to be evidence for hæmolysis; and Sjölin (1959) in an extensive review reported erythrocyte survival studies in four patients: the ^{51}Cr T$\frac{1}{2}$ was 9–10 days, and in two patients normal erythrocytes were also shown to have a greatly reduced survival.

The hæmolytic component in osteopetrosis seems largely to be the result of "hypersplenism" (see also p. 768), for transfused normal erythrocytes have been found to survive normally after splenectomy. More recently, Gamsu, Lorber and Rendle-Short (1961) have reported two further cases of "malignant" osteopetrosis in which there was evidence of marked hæmolysis—in one case the ^{51}Cr T$\frac{1}{2}$ was as short as 2·7 days.

POLYCYTHÆMIA

The possibility that latent hæmolysis might be a feature in some cases of polycythæmia vera has been considered from time to time.

Lawrence, Berlin and Huff (1953) obtained evidence which suggested that this might not be infrequent by showing that in five patients to whom ^{14}C-glycine had been administered the form of the disappearance curve of the labelled hæmin indicated the presence of short-lived erythrocytes as well as of some which survived for the normal length of time. In line with these observations are the data of Johnson, Hughes, Bird and Patrick (1957) who in seven patients found the mean ^{51}Cr $T\frac{1}{2}$ to be 19·9 days (normal mean, 25·4 days).

Cases of polycythæmia in which there is clinical evidence of hæmolysis are undoubtedly rare. Paschkis (1927) referred to a patient who was icteric. This probably in fact only occurs in developing myelosclerosis, as in the patient reported by Rau, Pulvertaft and Humble (1946).

MYELOSCLEROSIS

Many references to the occurrence of excess hæmolysis can be found in the large volume of literature now available on myelosclerosis.[1]

Hickling (1937), in a relatively early review, described seven personally studied patients; he observed raised serum-bilirubin levels in some of them and concluded that excess hæmolysis might be occurring. Edwards (1951) referred to two cases whose blood transfusion requirements were greatly reduced following splenectomy and Franks (1952) described a patient in whom excessive hæmolysis before operation was established by the finding of a shortened erythrocyte life-span (Ashby's method) and a raised excretion of fæcal urobilinogen. Mersky and Budtz-Olsen (1953) demonstrated in one patient the restoration to normal of the survival of transfused cells following splenectomy, and Loeb, Moore and Dubach (1953) and Green, Conley, Ashburn and Peters (1953) described patients with overt hæmolytic anæmia who were benefited by splenectomy. Hennemann, Kunz and Gillert (1953) reported two patients who had raised reticulocyte counts and increased osmotic fragility and in whom the direct antiglobulin reaction was positive. Four patients were mentioned by Verloop (1955): all showed increased fæcal urobilinogen excretion. Other patients showing signs of excessive hæmolysis and who were successfully treated by splenectomy were referred to in a discussion published in *Blood* (Cartwright and others, 1955).

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[1] Synonyms for myelosclerosis include the terms myelofibrosis, chronic non-leukæmic myelosis, osteomyeloreticulosis, osteomyelosclerosis, agnogenic myeloid metaplasia and primary myeloid metaplasia.

Since the above-quoted early reports larger series of patients have been described and there seems but little doubt that excessive hæmolysis often plays a role in the pathogenesis of the patients' anæmia, second only in importance to inadequate erythropoiesis. Overt hæmolytic anæmia, with clinical jaundice, high reticulocyte count, and increased osmotic fragility, etc., is, however, not commonly found, but some idea of its incidence may be found in papers mentioned in the following paragraph. Adding all the data together the incidence of overt hæmolytic anæmia appears to be in the region of 16%.

Thirteen patients were referred to by Richmond and Duncan (1956): four of them were considered to be suffering from symptomatic hæmolytic anæmia. Beyreder and Rieder (1956) reviewed 25 patients, three of whom showed signs of hæmolytic anæmia. Linman and Bethall (1957) studied 56 patients and reported that four of them showed signs of hypersplenism (hæmolysis). Bowdler and Prankerd (1961) referred to 16 patients, three of whom presented with hæmolytic anæmia. Pitcock, Reinhard, Justis and Mendelsohn's report (1962) was based on 70 patients, nine of whom showed signs of overt hæmolysis and underwent splenectomy. Finally, Bouroncle and Doan (1962) reviewed no less than 110 patients, 24 of whom were splenectomized for hypersplenism, having signs of hæmolytic anæmia and/or thrombocytopenic purpura.

The incidence of increased hæmolysis of a minor grade is certainly much higher than that for overt hæmolytic anæmia and it seems likely that, as in true leukæmia, a greater or lesser degree of shortening of erythrocyte life-span would be found to contribute to the anæmia of almost every case if followed to its termination. Studies with ^{51}Cr give some support for this contention.

Méeus-Bith and co-workers (1957) observed diminished ^{51}Cr $T\frac{1}{2}$ values in four out of six cases and Fudenberg and Mahoney (1958) obtained evidence of hæmolysis in 11 out of 28 patients. Nathan and Berlin (1959a) observed shortened ^{51}Cr $T\frac{1}{2}$ values in four out of five patients, Szur and Smith (1961) in 18 out of 19 patients and Nakai, Craddock and Figueroa (1962) in 14 out of 17 patients.

In several of these studies surface counting of the liver and spleen has been carried out in addition to measurement of blood radioactivity. Ultmann (1958) reported increased radioactivity over the spleen in one case and Nathan and Berlin (1959a) evidence for splenic sequestration in two patients. Szur and Smith (1961), who studied 19 patients in all and who found a slight to moderate diminution in erythrocyte life-span in 18 of them, obtained, however, evidence of an important degree of splenic sequestration in only four patients. Nakai, Craddock and Figueroa (1962), on the other hand, interpreted their data as showing suggestive or definite evidence of sequestration in all 14 patients whose ^{51}Cr $T\frac{1}{2}$ was reduced.

The above-mentioned studies leave little room for doubt that the enlarged spleen plays an important part in bringing about hæmolysis in many patients who suffer from myelosclerosis. This is also clearly demonstrated by the benefit often brought about by splenectomy in patients showing signs of overt hæmolysis (see p. 776). The reported differences in the occurrence of splenic sequestration probably depend on selection of patients for study as well as on differences in the interpretation of the radioactivity measurements.

However, despite its frequency hæmolysis is seldom very severe in myelosclerosis and the minimum ^{51}Cr T$\frac{1}{2}$ recorded for the patients described in the above-mentioned publications is 9·5 days, corresponding to a mean cell life-span of about 15 days. Lind (1960), however, in a single case report recorded an erythrocyte half-survival time of 8–9 days.

Serological Findings. The direct antiglobulin test seems almost invariably to be negative in myelosclerosis, and in none of the patients studied by the author and his colleagues did there appear to be an auto-immune element to their anæmia.

Clinical Course

Myelosclerosis runs a variable but as a rule a prolonged course. Where present, overt hæmolysis is most often seen towards the end of an illness which has already lasted some years. Then, after perhaps a long period of freedom from severe anæmia, the hæmoglobin level commences to fall and eventually transfusion becomes essential. After a variable length of time the intervals between transfusions progressively diminish and it becomes increasingly obvious that the transfused blood is surviving less and less well. It is in these patients that splenectomy is often carried out, usually as a last resort, and occasionally with considerable benefit (see p. 762). Less frequently, a hæmolytic element to the patient's anæmia is obvious at the time his illness is first diagnosed.

The relationship between spleen size and hæmolysis is uncertain. Those patients who show obvious evidence of hæmolysis generally have greatly enlarged spleens but massive splenomegaly can occur in patients without anæmia. Other factors than spleen size alone are clearly important.

Erythrocyte life-span and other data obtained in patients studied at the Postgraduate Medical School of London by the author and his colleagues are tabulated in Table 66. The clinical and hæmatological findings in two additional patients are summarized in the following case reports (Cases 20 and 21).

Table 66

Results of Erythrocyte Survival and Surface-Counting Studies in 19 Patients with Myelosclerosis

(Abridged from Szur and Smith, 1961, Table II)

Case No.	^{51}Cr T$\frac{1}{2}$ (Days)	Excess spleen counts	Excess liver counts	Spleen:liver ratio
		(Measurements at ^{51}Cr T$\frac{1}{2}$)		
1	32	260	200	1·9
2	19	500	250	1·1
3	21	300	200	1·7
4	18	500	200	1·2
5	16	300	200	1·0
6	17	500	200	1·6
7	16	450	280	0·9
8	16	700	200	2·0
9	15	800	200	1·7
10	12	300	200	...
11	14	400	200	1·3
12	19	400	200	1·2
13	20	950	200	2·0
17	12	650	200	1·6
18	16	400	200	1·3
19	17	400	200	1·6
Normal range	28 ± 2	<350	<200	

The patients' own erythrocytes were labelled with ^{51}Cr.

Case Report: Myelosclerosis and Hæmolytic Anæmia

Case 20. A married woman, M. W., aged 62, first attended Hammersmith Hospital in May 1960; she then gave a history of having occasional "blackouts" over a period of many years. She had become short of breath within the last 2 years, particularly during the last 6 months.

Physical Examination. She was slightly pale but not jaundiced. The spleen was enlarged to 15 cm. below the left costal margin and the liver was palpable 5 cm. below the right costal margin. The peripheral lymph nodes were not palpably enlarged.

Laboratory Findings. On admission, the hæmoglobin was 10·0 g. per 100 ml., PCV 31%, with 10·8% reticulocytes. The leucocyte count was 6,000 per cu. mm., with 75% neutrophils, and the platelet count 430,000 per cu. mm. Neutrophil alkaline phosphatase activity appeared to be normal.

Blood films showed a moderate degree of anisocytosis and anisochromasia, and marked microspherocytosis and polychromasia;

"pincered" cells were quite frequent (Fig. 193). About 6 erythroblasts per 100 leucocytes were present and also occasional metamyelocytes and myelocytes.

Osmotic fragility was markedly increased: lysis commenced in 0·80% NaCl; the MCF was 0·51% NaCl. After incubation at 37° C. for 24 hours, 12·5% lysis occurred in 0·85% NaCl. Autohæmolysis was greatly increased: at 48 hours there was 22·5% lysis, reduced to 11·2% in the presence of glucose.

The direct antiglobulin test was negative; serum bilirubin was 0·8 mg. per 100 ml., blood urea 35 mg. per 100 ml. and serum uric acid 7·6 mg. per 100 ml. Serum albumin was 4·4 g. and serum globulins 2·2 g. per 100 ml.; the electrophoretic strip showed a normal pattern.

Bone marrow. Puncture was attempted twice. On the first occasion little could be aspirated from the sternum; but on the second, very cellular marrow was obtained from the iliac crest. Erythropoiesis predominated; leucopoiesis appeared to be normal, and megakaryocytes were present in increased numbers. Numerous platelets could be seen in large clumps.

A diagnosis of probable myelosclerosis was made, but the apparent severity of the hæmolysis and the microspherocytosis were looked upon as unusual. The successful aspiration of cellular bone marrow from the iliac crest was interpreted as indicating that marrow sclerosis (if present) was patchy.

Further Course and Progress. The patient was seen as an out-patient from time to time over the next 3 years. Clinically, her general condition gradually deteriorated, and by August 1963 the spleen and liver had increased in size to 17 cm. and 8 cm. below the costal margins, respectively. She had also become more anæmic; the hæmoglobin had fallen to 6·5 g. per 100 ml. and the reticulocyte count had risen to 18·6%. Microspherocytosis continued to be a striking feature of the films.

The leucocyte count had ranged between 6,000 and 11,000 per cu. mm. over the 3-year period and up to 4% of myelocytes and occasional myeloblasts had been recorded. The platelet count was always raised; it had varied between 430,000 and 900,000 per cu. mm.

Osmotic-fragility studies carried out in June 1963 showed, as before, a marked increase; 5% lysis took place in 0·80% NaCl and the MCF was 0·525% NaCl. After incubation for 24 hours at 37° C., 27% lysis occurred in 0·85% NaCl. Autohæmolysis, too, remained markedly increased: 14·7% after 48 hours, which increased to 18·5% in the presence of glucose.

The survival of the patient's own erythrocytes was measured with ^{51}Cr in June 1963. The ^{51}Cr $T\frac{1}{2}$ was found to be markedly reduced (9·5 days) and significant excess radioactivity developed in the spleen; the spleen:liver ratio was 1·5 on the 10th day. Ferrokinetic studies utilising ^{59}Fe gave a $T\frac{1}{2}$ plasma clearance of 17 minutes (normal 60–120 minutes); utilization was, however, reduced (35%, normal >80%). Radioactivity due to ^{59}Fe was demonstrated in the spleen on the 5th day but not before this.

The above results were interpreted as showing that the erythrocyte life-span was markedly shortened, that hæmolysis was taking place to some extent in the spleen, and that the spleen was not an important site of extramedullary erythropoiesis. In view of these facts, and the clinical deterioration of the patient, splenectomy was advised: this was carried out by Mr. R. H. Franklin on the 30th August 1963. The spleen weighed 2,076 g.; it was deep plum in colour. Sections showed that the Malphighian follicles were widely separated and that the pulp cords were engorged with erythrocytes. Some foci of extramedullary hæmopoiesis were present.

The splenectomy was carried out without undue difficulty or hæmorrhage. Unfortunately, however, 36 hours after the operation the patient suddenly collapsed and she died a few hours later. Post-mortem examination showed the immediate cause of death to be hæmorrhage into the gastro-intestinal tract. The source of the bleeding could not, however, be found.

At necropsy the liver was found to weight 1,930 g. and sections showed foci of hæmopoiesis. The appearance of the bone marrow varied: in the vertebrae it was cherry-red in colour but it was more greyish in the iliac crest. Sections showed areas of hyperplasia as well as of hypoplasia. Megakaryocytes were especially conspicuous and there was a moderate increase in reticulin fibres. The findings were thought to be consistent with the diagnosis of myelosclerosis.

Summary and Conclusions. The patient suffered from myelosclerosis accompanied by an unusual degree of hæmolysis. Microspherocytosis was conspicuous in blood films. Death occurred soon after splenectomy.

Case Report: Myelosclerosis; Splenectomy with Good Response

Case 21. A man, J. D., aged 46, was admitted into Hammersmith Hospital for investigation in March 1963. He had been well until early in 1959; he then complained of heartburn and cramps in the legs. In May 1960 he was investigated in hospital and found to have a grossly enlarged spleen which extended into the right iliac fossa. In addition, small lymph nodes were palpable in the axillæ and groins. Blood examination showed a leuco-erythroblastic anæmia and a diagnosis of myelosclerosis was made. He received blood transfusions in March and April 1960 and his spleen was irradiated in June and July 1960 (18 treatments of 25 r. each). He subsequently was treated with prednisone but nevertheless needed to be transfused at intervals and these were continued until his admission to Hammersmith Hospital.

Physical Examination (March 1963). His general condition appeared to be good and he did not appear to be markedly anæmic. The spleen was very large and extended into the right iliac fossa and the liver could be palpated 3 cm. below the right costal margin.

Laboratory Findings. On admission the hæmoglobin was 7·3 g. per 100 ml., erythrocyte count 2,200,000 per cu. mm., PCV 24·5%, with 3·6% reticulocytes. The leucocyte count was 8,000 per cu. mm., with 82% neutrophils and 2% myelocytes; the platelet count was 57,000 per cu. mm. Stained films showed marked anisocytosis and poikilocytosis with many oval and elliptical cells and some macrocytes (Fig. 194). A few erythroblasts were present and some hypersegmentation of neutrophils. The neutrophil alkaline phosphatase was markedly raised (204 units). The blood picture was thought to be characteristic of myelosclerosis with probable secondary folic-acid deficiency.

A series of blood counts carried out within a period of 1 month showed that the hæmoglobin was approximately steady at 7–8 g. per 100 ml., the reticulocyte count varied between 2·9 and 9·0%, the leucocyte count between 9,000 and 18,000 per cu. mm. and the platelet count between 25,000 and 68,000 per cu. mm.

Serum bilirubin was 0·9 mg. per 100 ml., serum uric acid 7·9 mg. per 100 ml., serum albumin 3·8 g. per 100 ml. and serum globulins 2·8 g. per 100 ml., with a normal pattern on electrophoresis. The blood urea was 24 mg. per 100 ml. The urine was normal.

The direct antiglobulin test was negative. The serum folate was subnormal (1·7 μmg. per ml.).

Bone-marrow aspiration was attempted; no marrow fragments were aspirated and few free marrow cells were present. Amongst them, however, were hypersegmented neutrophils and giant metamyelocytes.

The survival of the patient's own erythrocytes was studied using ^{51}Cr: the ^{51}Cr T$\frac{1}{2}$ was 13 days and there was a small excess splenic uptake of radioactivity. The blood volume was above normal (100·8 ml. per kg.), erythrocyte volume subnormal (27·1 ml. per kg.) and plasma volume markedly increased (73·7 ml. per kg.).

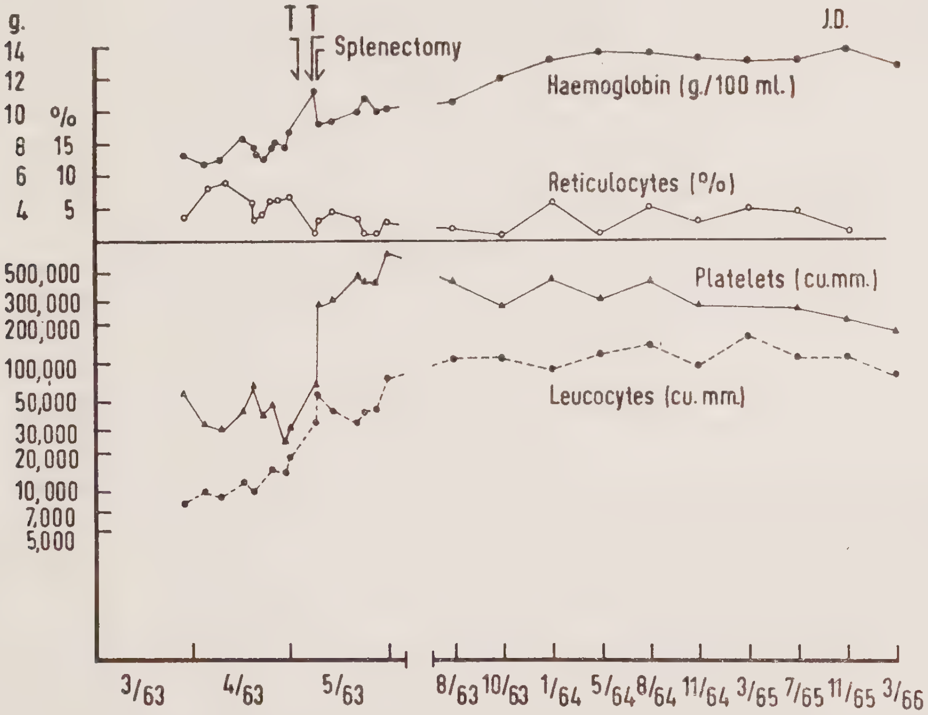

FIG. 196. Hæmatological data in a patient suffering from myelosclerosis and excessive hæmolysis (Case 21). Splenectomy was followed by considerable benefit. T = blood transfusions.

Further Course and Progress: Splenectomy. Because the spleen was very large and causing abdominal discomfort and because there was evidence of a reduced erythrocyte life-span, splenectomy was advised, and this was carried out by Mr. T. Menzies on the 7th May 1963. The spleen weighed 3,396 g. and measured 32 × 19 × 11 cm. The cut surface was uniformly plum coloured. Sections showed that the Malpighian follicles were small and widely separated and that there was marked fibrosis of the pulp in addition to areas of active hæmopoiesis and an increase in hæmosiderin. The appearances were considered to be consistent with those of myelosclerosis.

The patient recovered well from the operation and left hospital 23 days later. No further transfusions have been required and subsequent progress has been good (Fig. 196). The patient returned to full-time work and 2½ years later still reported that he felt very well. The liver increased in size soon after splenectomy and by August 1963 it had reached 12 cm. below the right costal margin; it remained at this size subsequently.

Blood Picture Post-Splenectomy. As expected, major changes followed removal of the spleen (Fig. 195). The hæmoglobin rose steadily and has been maintained at more than 13 g. per 100 ml. for the last 2 years. The platelet count rose sharply and reached a maximum of 795,000 per cu. mm. 3 weeks post-splenectomy; since then it has been slowly subsiding. The leucocyte count rose sharply, too, and has been maintained for 2 years at or slightly above 100,000 per cu. mm., with up to 90% of neutrophils. Many erythroblasts have been present in the blood also, in numbers varying from 25,000 to 50,000 per cu. mm.

Conclusion and Summary. The patient had typical myelosclerosis; there was evidence of a moderate increase in hæmolysis and repeated transfusions were necessary. Splenectomy was followed by a remarkably complete clinical remission which has so far lasted for 2½ years.

HÆMATOLOGICAL FINDINGS IN HÆMOLYTIC ANÆMIA IN MALIGNANT DISEASE OF THE LYMPHO-RETICULAR SYSTEM AND IN THE MYELOPROLIFERATIVE DISEASES

Blood Picture

The blood picture reflects both the nature of the underlying disease and the hæmolysis which accompanies it. Pointers to hæmolysis include auto-agglutination, spherocytosis and erythrocyte distortion and fragmentation. Regular anisocytosis and poikilocytosis are more likely to be consequences of disordered erythropoiesis than hæmolysis. The degree of polychromasia indicates the intensity of the reticulocyte response to the hæmolysis, which is often less in proportion to the patient's anæmia than in the "primary" hæmolytic anæmias. This is a reflection of the involvement of the bone marrow in the underlying disease process.

Erythroblasts are commonly met with in the peripheral blood in leukæmia and myelosclerosis and their presence denotes extramedullary hæmopoiesis and not necessarily very active erythrocyte regeneration. Megaloblastic change due to folic-acid deficiency has been occasionally observed as a complication of the secondary hæmolytic anæmias under discussion, as for instance in the case of chronic lymphocytic leukæmia accompanied by auto-immune hæmolytic anæmia described by Chanarin, Dacie and Mollin (1959).

Auto-agglutination when present is suggestive of an auto-immune origin for the hæmolysis (Fig. 198); if due to cold agglutinins it will disperse on warming the blood to 37° C.

Spherocytosis is particularly found where warm auto-antibodies are present, *e.g.*, in chronic lymphocytic leukæmia etc. (Figs. 183 and 192), but it may be obvious in myelosclerosis also where there is no evidence of auto-antibody formation; it is not usually an obvious feature in the hæmolytic anæmia accompanying Hodgkin's disease. In myelosclerosis the spherocytes are not infrequently slightly oval or elliptic in shape (Fig. 193).

Erythrocyte Osmotic and Mechanical Fragility. The osmotic and mechanical fragilities are both increased in the presence of spherocytosis, but the correlation between morphological change and fragility increase is not always a very close one.

Schilling (1952) studied the mechanical fragility of blood in leukæmia and found that this was significantly increased in a series of 47 leukæmic patients compared with a similar number of controls (mean values 6·5% and 4·4%, respectively). This series included 12 cases of acute leukæmia (mean 7·0%), but only one patient had an overt hæmolytic anæmia.

Bakker-v. Aardenne, Verloop and van Boetzelaer (1958) carried out an extensive investigation, 148 cases of "idiopathic" and symptomatic hæmolytic anæmia being studied. In Hodgkin's disease they found that the osmotic fragility of fresh blood was normal in 80% of 26 patients and mechanical fragility normal in all. But both tended to increase outside the normal range on incubation, mechanical fragility being abnormal in 90%. The values tended to become normal after the removal of leucocytes or substitution of isotonic phosphate buffer at pH 7·5 for plasma. In cases of chronic leukæmia (lymphocytic or granulocytic) these authors found that the osmotic and mechanical fragilities were increased in 80% of cases, but in myelosclerosis, although the osmotic fragility was usually increased, mechanical fragility tended to be normal. The significance of these observations is uncertain; they could not be clearly correlated with the presence or absence of hæmolysis.

Troup, Swisher and Young (1960) studied the rate of hæmolysis of sterile defibrinated blood which was incubated for 48 hours at 37° C. in a large series of patients suffering from leukæmia and allied disorders. The blood of 15 out of 25 patients with acute or subacute myeloid leukæmia showed increased autohæmolysis which in most instances was reduced substantially in the presence of excess glucose. In contrast, autohæmolysis was normal in 10 patients with chronic granulocytic leukæmia. In myeloid metaplasia (myelosclerosis), on the other hand, autohæmolysis was often increased and the rate of hæmolysis was not generally diminished in the presence of excess glucose; in some cases it was in fact increased. Ten cases of auto-immune hæmolytic anæmia accompanying chronic lymphocyte leukæmia were also studied. Autohæmolysis was generally increased but this became less when the patient went into remission. The addition of glucose usually had little effect on the increased hæmolysis.

Crosby and Benjamin (1955, 1957) (see p. 45, Volume I) observed a peculiar type of increased autohæmolysis in some cases of leukæmia or disseminated neoplastic disease. Crosby, Vullo and Garriga (1961) have more recently reported their results in a series of 55 cases of acute leukæmia. Increased lysis was noted in 20 out of 54 tests (37%) using heparin as anticoagulant and in 32 out of 44 tests employing defibrinated blood. But there seemed to be no correlation between the rate of lysis and the type of leukæmia, the clinical state of the patient or the prognosis, or between in-vitro lysis and in-vivo hæmolysis as measured with ^{51}Cr, or between the rate of lysis and the patient's blood picture.

Hæm Metabolism

The *serum-bilirubin* level is seldom raised substantially in the types of hæmolytic anæmia under discussion unless the rate of hæmolysis is markedly raised; often the level is well within the normal range. This is not to say that abnormal levels of serum bilirubin sufficient to cause clinical jaundice do not sometimes occur, but amounts in excess of 5 mg. per 100 ml. or the presence of substantial amounts of conjugated pigment are suggestive of the liver being involved in the underlying disease process.

Fæcal-urobilinogen levels vary from within the normal range to values well in excess of normal. However, if excretion is related to total circulating hæmoglobin it will generally be found to be raised. Moreover, in cases where there is much ineffective erythropoiesis, as in erythræmic myelosis, the excess of pigment excretion can frequently be demonstrated to be out of proportion to the demonstrable shortening in erythrocyte life-span (see p. 737).

PATHOLOGY AND PATHOGENESIS OF THE INCREASED HÆMOLYSIS IN HODGKIN'S DISEASE, LEUKÆMIA, RETICULOSARCOMA AND MYELOSCLEROSIS, ETC.

The numerous studies which have been cited in earlier pages of this chapter indicate that excess hæmolysis is frequently an important factor in the pathogenesis of the anæmia accompanying many types of myelo- or lympho-proliferative disease. Occasionally hæmolysis is the dominant mechanism, but it probably plays a greater or lesser role in almost every case at some time in the course of each patient's illness. Of course other mechanisms of anæmia are important; sometimes they are of supreme importance. They include replacement of bone marrow by tumour, interference by one means or another with erythropoiesis, hæmorrhage into or around the tumour(s) and external hæmorrhage. These latter mechanisms certainly must not be forgotten. However, a consideration of them in detail is beyond the scope of this book.

How is the excess hæmolysis brought about? There are without doubt several mechanisms: these include the effects of auto-antibodies, increased erythrophagocytosis, hypersplenism, general reticulo-endothelial cell hyperplasia, the consequences of interference with erythropoiesis, and, less tangibly, the effects of general toxæmia. Early views on these problems are summarized in Price and Greenfield's (1958) review.

Auto-Antibodies. The way in which auto-antibodies bring about hæmolysis has been discussed at some length in Chapter 11 (Volume II) of this book, and there is no reason to suppose that the general effects of the antibodies differ between "idiopathic" and secondary cases. They show many similarities, too, in their laboratory reactions (see Tables 63 and 64). Even when present, however, the other hæmolytic mechanisms which are presumed to operate in the many cases in which auto-antibodies cannot be demonstrated (see below) probably also play a part.

Erythrophagocytosis. This is often a striking phenomenon in the vicinity of nodules of tumour tissue. This is, for instance, true of Hodgkin's disease tissue in the spleen, so much so that the nodules may be surrounded by a wide cuff of phagocytes containing yellow pigment (hæmosiderin). This can be a striking feature and it has been remarked upon from time to time from the earliest reports of hæmolysis in association with Hodgkin's disease (Netousěk, 1922–3; Davidson, 1932 (his Case 2); Schiappoli, 1934; Marchal, 1947; Dacie, 1954 (Case 19)) (Fig. 197). The mechanism of this local hæmolysis is not clear but it seems possible in these cases that potentially lytic metabolites are generated by the growing tumour tissue and that these metabolites damage the patient's erythrocytes as they circulate in close contact with the pathological tissue, as may happen when the spleen (or bone marrow) is infiltrated. Another possibility is that of continuous leakage of erythrocytes into the tissues surrounding the tumour as the result of destruction of blood vessels, and excessive vascular permeability. A further possibility is that the local phagocytic cells are stimulated by metabolites derived from the growing tumour tissue. These possibilities and the lytic effects that tumour cell extracts are known to have *in vitro* are further discussed on p. 793.

Erythrophagocytosis is also seen in the vicinity of reticulo-sarcomatous tissue growing in lymph nodes, the lymph sinuses being then particularly rich in erythrocyte- and hæmosiderin-laden phagocytes. In some of these cases at least this could be the effect of the production of auto-antibody locally within the infiltrated node; alternatively, this could be the result of "non-specific" stimulation of phagocytes by the tumour.

"Hypersplenism." Another factor which has often been considered to be important is "hypersplenism." It seems probable that any disease process which causes splenic enlargement, with increased opportunities for erythrostasis and hyperplasia of the phagocytic cells, will bring about an increase in hæmolysis. It is probably not necessary to postulate any pathological alteration in phagocytic cells for this to happen. A simple increase in numbers of phagocytic cells may suffice (see also p. 929).

Hypersplenism has usually been invoked as a hæmolytic mechanism only in cases in which signs of hæmolysis are associated with thrombocytopenia, *e.g.*, by Bouroncle and Doan (1962) in cases of myelosclerosis. This may, however, be a too restricted viewpoint. A very large spleen is also important in relation to anæmia because of the large amount of blood it may contain; as pointed out by Fudenberg and Mahoney (1958) a low packed cell volume (in myelofibrosis) is in part due to pooling of erythrocytes in the spleen.

The spleen, too, is of particular importance in cases where auto-antibodies are being formed. Not only is the spleen a site of antibody formation but it is the most important site for the destruction of erythrocytes coated by incomplete antibodies, as the occasional remarkable remissions in the auto-immune hæmolytic anæmia accompanying cases of chronic lymphocytic leukæmia or other lymphomas bear witness (see p. 775). Although splenomegaly in these patients has been reported as being disproportionately great (Berlin, 1951; Rosenthal *et al.*, 1955), this has not been the experience of all workers (Freymann, Burrell and Marler, 1958); but the volume of spleen available for sequestering erythrocytes is no doubt more important in relation to hæmolysis than the enlargement due to the tumour tissue.

Deranged Erythropoiesis. A characteristic feature in leukæmia, particularly if advanced, and in Hodgkin's disease, reticulosarcoma with bone-marrow involvement or myelosclerosis, is the wide variation in size and shape of the patient's erythrocytes. Not infrequently microcytes are present suggesting that fragmentation is occurring, too. The mechanisms producing these changes are ill-understood. Presumably they originate in intrinsically disturbed erythropoiesis, as in cases of leukæmia and erythræmic myelosis, as well as from interference with the availability of essential metabolites by the presence of tumour tissue in the bone marrow. The not infrequent occurrence of overt folic-acid deficiency is evidence of this.

An appreciable degree of spherocytosis may be seen in some cases and this change, too, is probably associated with a reduced

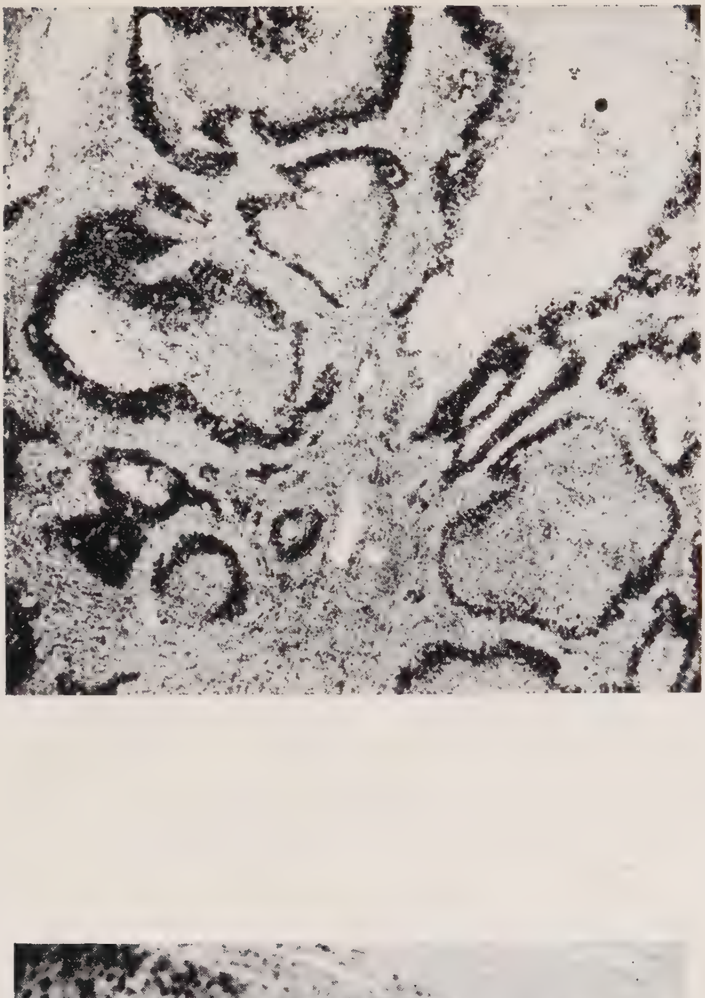

FIG. 197. Photomicrograph of a section of the spleen of a patient suffering from Hodgkin's disease complicated by hæmolytic anæmia (Case 19 of Dacie, 1954). The nodules of tumour tissue are surrounded by a dense cuff of iron-containing pigment. Perls's reaction. × 17 and × 50.

To face p. 768.

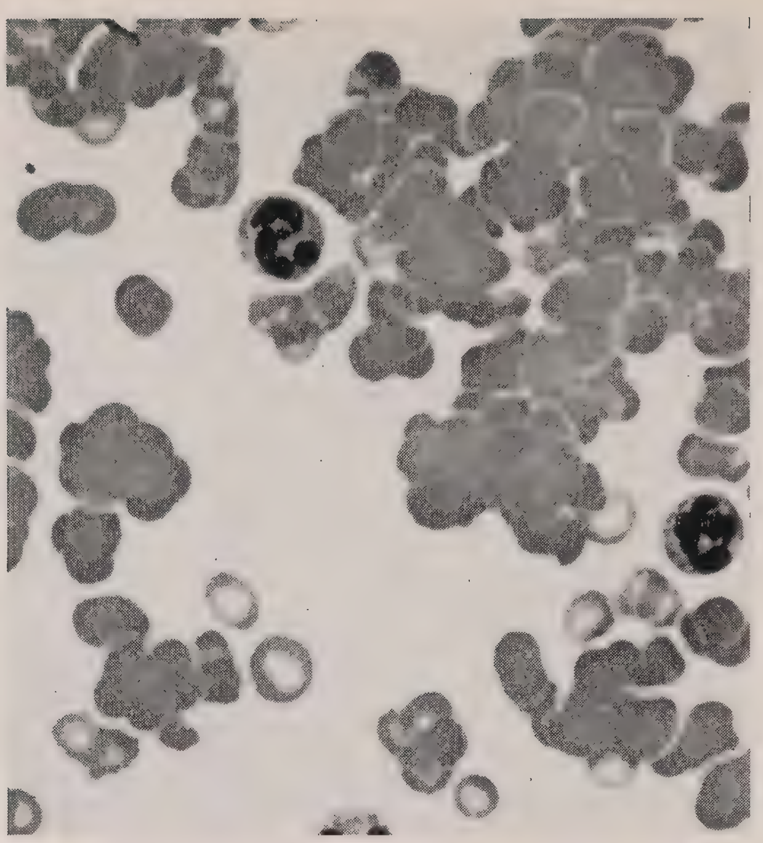

Fig. 198. Photomicrograph of a blood film of a patient suffering from a malignant lymphoma complicated by auto-immune hæmolytic anæmia (Case S.B. of Table 64). Auto-agglutination is conspicuous. × 700.

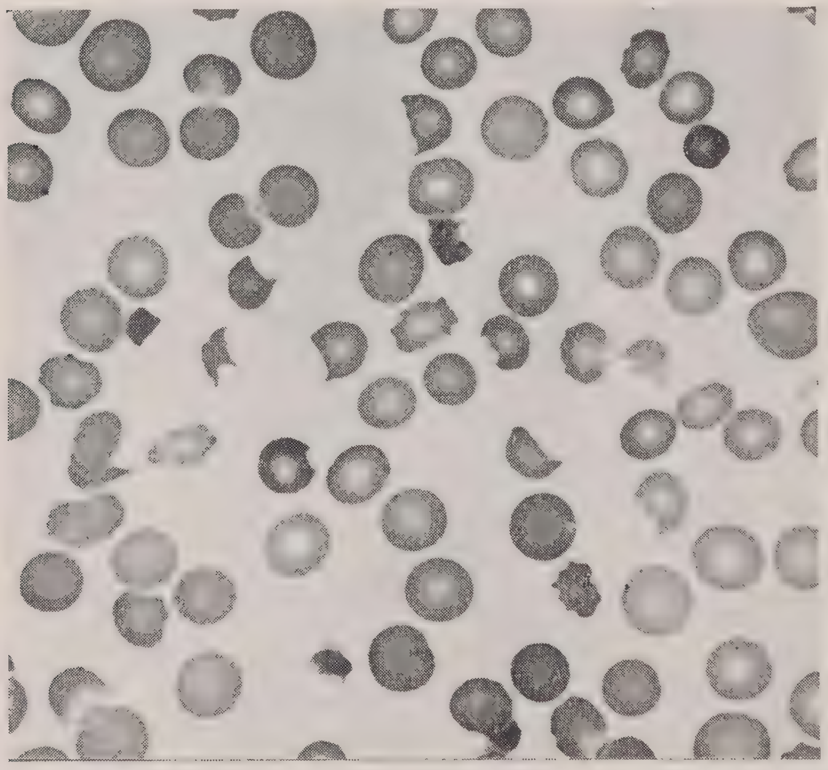

Fig. 199. Photomicrograph of a blood film of a patient suffering from disseminated carcinoma of the stomach (Case 22 of Dacie (1954)). Many contracted and distorted and "burr" cells are present; and there is a tendency to spherocytosis. × 700.

erythrocyte survival The mechanisms underlying the spherocytic change are uncertain: in some cases it can be clearly correlated with auto-antibody formation, as in "idiopathic" auto-immune hæmolytic anæmia, but in others this cannot be the explanation, *e.g.*, in the atypical chronic granulocytic leukæmia reported by Darte, Dacie and McSorley (1954). Spherocytosis has been known for a long time to be relatively frequently seen in myelosclerosis (Vaughan and Harrison, 1939) (Fig. 193), but whether the change can be explained by increased erythrostasis in the spleen or whether it is a reflection of extramedullary erythropoiesis or of a peculiar type of erythropoiesis occurring in the marrow and elsewhere is uncertain.

The net result of deranged erythropoiesis is the production of less than perfect erythrocytes, some at least of which have shortened life-spans. The relative parts played by intrinsic defects secondary to dyshæmopoiesis and extrinsic hæmolytic mechanisms must vary from case to case. Probably both factors are important in most cases but not necessarily in the same proportion. Fudenberg and Mahoney (1958), who carried out cross-transfusion experiments in patients with myelofibrosis, concluded that intrinsic factors were the more important in the cases they studied.

As already mentioned (p. 765), studies of erythrocyte fragility *in vitro*, and of autohæmolysis, do not generally correlate well with clinical evidences of hæmolysis, but in individual cases increases in osmotic and mechanical fragility have been noted at times when hæmolysis has been accelerated (Craig, Waterhouse and Young, 1952).

General Toxæmia and the "Hæmopathic State"

There is clinical evidence that the severity of a patient's anæmia often varies with the fluctuations in the intensity of the "primary" disease process. In Hodgkin's disease, in particular, patients usually become more anæmic during the febrile phases of a Pel-Ebstein cycle (Fig. 180, p. 722). Such increases in anæmia may in part be due to exacerbations in hæmolysis. The term "hemopathic hemolytic anemia" has been used, too, by Wasserman and his co-workers (1955) and Dameshek and Gunz (1958) to describe the hæmolysis in very sick patients in whom there appears to be no very particular mechanism for it.

Azotæmia, also, must not be forgotten as a potential cause of hæmolysis (see p. 838). This clearly could be a significant factor in cases of multiple myeloma (Cline and Berlin, 1962).

TREATMENT

Treatment will be considered under five headings: transfusion; steroid therapy; therapy with anti-metabolite or immuno-suppressive drugs; radiotherapy, and splenectomy.

Transfusion

The limitations of blood transfusion in the treatment of hæmolytic anæmia brought about by mechanisms other than intrinsic erythrocyte abnormalities need no emphasis: the survival of the transfused normal cells is often no better than that of the patient's own cells and may even in fact appear to be worse (p. 675, Part II). But, as in "idiopathic" cases of auto-immune hæmolytic anæmia, transfusion may be life-saving by buying time for more specific treatment to become effective (Fig. 184). Despite the transfused blood's subnormal survival transfusion seems rarely to do positive harm, even in cases of auto-immune origin where excess free antibody is present in the patient's serum. It should be mentioned, however, that transfusion has been considered in certain cases as a possible activator of the hæmolytic process (Craig, Waterhouse and Young, 1952).

Steroid Therapy

Adrenocorticotrophic hormone (ACTH), and cortisone and prednisone etc., have been extensively used and a greater or lesser degree of suppression of hæmolysis has often been observed; and in some cases also definite control or regression of the underlying disease.

The first reports of successful therapy date from 1951. Then, Dameshek, Rosenthal and Schwartz described the effects of ACTH therapy in three cases. Two patients with lymphosarcoma were greatly benefited; the hæmoglobin rose in both, the serum-bilirubin level fell as did the titre of abnormal agglutinins. A third patient, suffering from chronic lymphocytic leukæmia, reacted less favourably. Craig, Waterhouse and Young (1952), however, reported a good response in a case of chronic lymphocytic leukæmia and Dacie (1954, Case 21) an excellent remission in a patient who had an underlying reticulosarcoma and had developed cold auto-antibodies.

Many reports of successful steroid therapy have been published subsequently; most, however, deal with chronic lymphocytic leukæmia or other malignant lymphomas complicated by hæmolysis of auto-immune origin. Matthias (1964a, b) has recently demonstrated that steroid therapy can prolong erythrocyte life-span in both immune and non-auto-immune types of hæmolysis. With steroids the effect is often immediate and dramatic, and independent of any anti-tumour effect, as compared with chemotherapy where benefit is more gradual and seemingly related to an anti-tumour effect.

As there are some differences in response to steroids in the different disorders so far considered in this Chapter, each disease or group of diseases will be dealt with separately.

Hodgkin's Disease. Steroids normally produce only limited benefit at the best. They are perhaps of most value in exceptional situations, as, for example, in the patient of Wasserman and his co-workers (1955) who had an auto-immune hæmolytic anæmia, and in the patient described by Bove (1956) who had erythroblastopenia. Bousser and his colleagues (1961) also referred to two patients with Hodgkin's disease who responded to steroids. Both gave positive direct antiglobulin tests.

Acute and Chronic Myeloid Leukæmias. Here there seems little evidence that steroids have any effect in controlling hæmolysis.

Chronic Lymphocytic Leukæmia, Giant Follicle Lymphoma and Lymphosarcoma. Most of the serious hæmolytic anæmias in this group of patients seem to have an auto-immune component and it appears that such patients may respond as well and as completely, from the point of view of suppression of excess hæmolysis, as do patients with "idiopathic" auto-immune hæmolytic anæmia.

Dameshek and Komninos (1956) reviewed a total of 19 cases. Ten out of 17 patients with chronic lymphocytic leukæmia experienced an "excellent" or "good" initial response and in five more there was a "slight" or "fair" response. These results are almost as good as can be expected in patients with the "idiopathic" disease. Seaman, Koler, Pirofsky and Osgood (1958) referred to severe hæmolysis as developing in 50 out of 200 consecutive patients with chronic lymphocytic leukæmia. They stressed that steroid therapy was the treatment of election, and that the outlook for the patient with overt hæmolysis was less good than in its absence. They also made the point that patients with evidence of hæmolysis but without positive antiglobulin tests might also be benefited by steroid therapy.

Lichtman and his co-workers (1957) reported on the use of prednisone in seven patients with chronic lymphocytic leukæmia or lymphosarcoma and in two with myelomatosis: eight of them were improved. They stressed, however, that large doses (up to 80 mg. daily) might be necessary in some cases and referred to the risk with high-dose therapy of infections complicating treatment (see Case 15, p. 735).

Chanarin, Dacie and Mollin (1959) described an exceptional patient in whom hæmolysis was associated with folic-acid deficiency. This patient responded well to prednisone (and to folic acid). Another series of patients in the control of whose anæmia steroids had proved of benefit was described by Troup, Swisher and Young (1960).

Geller (1964) reported on the exceptionally interesting history of a patient who had, at the time of reporting, experienced a 9-year remission, both with respect to chronic lymphatic leukæmia and hæmolytic anæmia, after prolonged treatment with ACTH.

Lymphosarcoma, Reticulosarcoma and Macroglobulinæmia. Where the complicating hæmolytic anæmia is auto-immune in type remission may follow steroid therapy, *e.g.*, as in the case of reticulosarcoma described by Dacie (1954, Case 21) and the two cases of lymphosarcoma reported by Dameshek and Komninos (1956) (see also Fig. 186). Good responses, too, have been observed in macroglobulinæmia (Bousser and Boivin, 1957).

Myelosclerosis. Steroids are at the best of limited value in the control of hæmolysis in myelosclerosis. None of the patients studied by the author and his colleagues has shown unequivocal benefit, and on the whole the author feels that steroid therapy is best avoided.

Anti-Metabolite, Immuno-Suppressive and X-Ray Therapy

It is reasonable to suppose that treatment of the underlying disease should be of benefit ultimately to a complicating hæmolytic anæmia, and sometimes this appears to be so.

Favourable responses have been reported by Feuchtinger (1942) (X-ray therapy in chronic lymphocytic leukæmia), Brown and Meynell (1949) (X-ray therapy in Hodgkin's disease), Bremy (1951) (nitrogen-mustard therapy in Hodgkin's disease), Wasserman and his co-workers (1955) (nitrogen-mustard and X-ray therapy in Hodgkin's disease), Bousser and co-workers (1961) ("Caryolysine" in Hodgkin's disease) and Djaldetti, de Vries and Levie (1962) (X-ray therapy in five patients with chronic lymphocytic leukæmia). It should be added that X-ray therapy (and alkylating agents) have been blamed for precipitating attacks of hæmolysis in certain patients (Marchal and Duhamel, 1950; Rosenthal *et al.*, 1955; Lewis, Schwartz and Dameshek, 1966), a possibility which is difficult to prove; and also that by no means every patient will respond favourably to treatment.

Anti-metabolites and nitrogen mustards and allied drugs suppress immune mechanisms as well as controlling neoplastic myelo- and lympho-proliferation. In theory at least, therefore, their immuno-suppressive action should be particularly beneficial to patients with auto-immune hæmolysis.

Splenectomy

It is convenient to consider the results of splenectomy under three disease headings: Hodgkin's disease; leukæmia, reticulosarcoma and allied disorders; and myelosclerosis. Each group has a large literature.

Hodgkin's disease. As referred to earlier, some of the earliest reports on Hodgkin's disease and hæmolytic anæmia referred to patients who had been operated on for splenomegaly or "splenic anæmia" and in whom histological examination of the excised spleen showed Hodgkin's disease. Subsequently, splenectomy has been carried out deliberately on patients suffering from severe hæmolysis, with in some cases moderate or even dramatic improvement.

Singer and Dameshek (1941) reported improvement for 5 months in one patient. Stats, Rosenthal and Wasserman (1947) referred to two patients: one did not respond, but the other experienced a remission of anæmia which lasted until her death 7 months later. Scott (1949) recorded a 4½-year remission and Brown and Meynell (1949) some improvement in a further case.

Williams, Andrews and Zanes (1951) referred to 11 splenectomies for hypersplenism in Hodgkin's disease; the blood picture of nine of these patients was improved. Fisher, Welch and Dameshek (1952) mentioned two patients, in one of whom the anæmia became less severe after splenectomy. Sykes and his colleagues (1954), in reviewing the literature, stated that ten out of 16 patients with Hodgkin's disease and hæmolytic anæmia had been improved by splenectomy. More recently, Rousselot, Rella and Rottino (1962) reported upon the outcome of the operation in 14 patients who had been splenectomized out of a total of 347 cases of Hodgkin's disease. One patient died immediately postoperatively and five more within 6 weeks, and the average survival was only 5·9 months. Rousselot and co-workers concluded that only a small proportion of patients was suitable for splenectomy but that in some of these, suffering from secondary hypersplenism, a reduction in transfusion requirements might be achieved.

Schultz, Denny and Ross (1964) referred to 10 patients with Hodgkin's disease who had undergone splenectomy for overt hæmolysis: four of them responded well to the operation and had remissions lasting between 3½ and 25 months.

The above reports suggest that the possibility of improvement following splenectomy in Hodgkin's disease should not be overlooked. Nevertheless, the successful operations no doubt tend to be reported rather than the failures, and the author finds it impossible to look upon splenectomy in Hodgkin's disease with any enthusiasm. Although anæmia may improve temporarily in some patients, such improvement seems to have little effect as a rule on the downhill course of the disease, particularly when the operation is carried out on patients whose disease is advanced and widespread. Perhaps the operation is only really worth while in the exceptional patient who develops marked hæmolysis early in the course of his or her illness (but see Case 14, p. 724).

Leukæmia

Chronic or Subacute Myeloid Leukæmia. Splenectomy has been undertaken from time to time in various types of myeloid leukæmia with apparent benefit to some of the patients. Brill (1924), for instance, reported temporary improvement in a patient probably suffering from myeloblastic leukæmia or erythræmic myelosis and Ferrata and Fieschi (1938) concluded that there was a special indication for splenectomy in leukæmic patients with severe hæmolytic anæmia and thrombocytopenia but without myeloblastic proliferation in the bone marrow. Other authors, such as Gasser (1946), have considered splenectomy to be inadvisable on the grounds that the operation removed an organ which might exert a humoral inhibitory effect on the bone marrow. Jonsson, Hansen-Pruss and Rundles (1960), on the other hand, have described a patient with chronic myeloid leukæmia in whom splenectomy was remarkably successful, being followed by a period of 30 months during which time no transfusions were required.

Berlin's (1951) observations were based on a study of six patients. Two of them underwent satisfactory remissions and remained in good health for almost 2 years after the operation, but the results were less satisfactory in the others. Although in each case there was an improvement or restoration to normal of the survival of transfused normal erythrocytes, the patients' general condition deteriorated for one reason or another. Berlin considered (1) that splenectomy should only be undertaken in cases where there was clear evidence of excess hæmolysis and (2) that the operation should be performed as early as possible in the course of the disease. He also considered that the leukæmia should be brought into remission if possible by chemotherapy or irradiation before the operation was attempted. He reviewed, too, the evidence as to whether splenectomy increased the chances of a fatal myeloblastic crisis and decided that this was inconclusive.

Splenectomy seems to have been less frequently undertaken in recent years. Fisher, Welch and Dameshek (1952) mentioned one patient with monocytic leukæmia who was improved for 3 months, and stated that another patient with myeloid leukæmia derived no benefit. The present author has studied two patients before and after splenectomy. In both minor benefit ensued but both died of pulmonary infection not long after the operation. The course of one of these patients was described by Darte, Dacie and McSorley (1954): the history of the other patient, who had a myelo-monocytic leukæmia and a positive antiglobulin reaction, is summarized on p. 738 (Case 16). The results of the operation in both these patients are hardly encouraging.

There is no disguising the fact that the morbidity and mortality associated with splenectomy in myeloid leukæmia of all types are high, and that even if hæmolysis can be controlled by removing the spleen the underlying disease remains untouched. It is impossible to contemplate splenectomy in the myeloid leukæmia group of disorders with any enthusiasm.

Chronic Lymphocytic Leukæmia and Allied Lymphomas. The chances of improvement following splenectomy are much better in chronic lymphocytic leukæmia than in the myeloid leukæmias. This is probably because many of the severe hæmolytic anæmias in the former group have an auto-antibody component. If this is so, provided the leukæmia itself is in an early stage of evolution, there is the possibility of a dramatic success. Nevertheless, the operation may fail to alleviate the hæmolysis (as it may in cases of "idiopathic" auto-immune hæmolytic anæmia) and it can hardly be recommended late in the course of the disease. The post-operative mortality and morbidity risk are then high, the prevention of infection being a particularly serious problem.

Hutt (1950) reported striking benefit in one patient with chronic lymphocytic leukæmia and Evans and Doan (1951) and Hagen and Watson (1951) each referred to series of patients with giant follicle lymphoma or chronic lymphocytic leukæmia who were improved by splenectomy. Whitelaw (1957) referred to seven cases of leukæmia or lymphoma out of a total of 450 in which splenectomy had been undertaken: two of these patients had lymphosarcoma and both derived some benefit from the operation. Videbaek (1962) reported on five patients with chronic lymphocytic leukæmia and auto-immune hæmolytic anæmia who were submitted to splenectomy. All were benefited to a greater or lesser extent and the best results were seen in the two patients whose spleens contained many plasma cells and reticulum cells and were thought to be a source of antibody.

Strumia and his co-workers (1962) similarly reported that nine patients with chronic lymphocytic leukæmia submitted to splenectomy benefited: in one patient (their Case 12), who had a very severe auto-immune hæmolytic anæmia, the response was dramatic; in another (their Case 8), whose erythrocyte survival had been measured, splenectomy was followed by a marked reduction in the early loss of circulating radioactivity, suggesting that erythrocytes had previously been trapped in the enlarged spleen.

Schultz, Denny and Ross (1964) reported on the results of splenectomy in 11 patients with chronic lymphocytic leukæmia complicated by hæmolytic anæmia: the response in seven was said to be good and to last from 2 to 18 months.

Dameshek and Gunz (1964a) quoted a case where splenectomy was followed not only by cure of the hæmolytic anæmia but also by a gradual and complete disappearance of the signs of leukæmia; but they (1964b) also mentioned, in relation to splenectomy in patients whose hæmolytic anæmia seemed to have a definite auto-immune component, that it is usually extremely difficult to decide whether or not to undertake the operation, an opinion with which the present author agrees. Clearly all other means of therapy should be utilized, and exhausted, before the drastic procedure of splenectomy is recommended.

Myelosclerosis. Splenectomy has been carried out quite frequently in myelosclerosis and brilliant results have sometimes been obtained. Nevertheless, despite these occasional successes, its value and justification have been a matter of controversy. The operation entails considerable risk for the patient and it should certainly not be lightly advised. Moreover, as in cases of genuine leukæmia for which splenectomy has been carried out, successful operations tend to be reported more frequently than unsuccessful ones.

Splenectomy has been undertaken not only because of hæmolysis and/or signs of hypersplenism but also to relieve the discomfort arising from the presence of a very large spleen. The case reports which are mentioned below refer to splenectomy undertaken primarily for hæmolysis and any benefit mentioned refers to a lessening of the signs of hæmolysis and/or a reduction in transfusion requirements.

Edwards (1951) mentioned two patients, both of whom were improved by splenectomy and Franks (1952) reported a dramatic improvement in a single case. Mersky and Budtz-Olsen (1953) described a partial remission in one case, with a normal survival of transfused normal erythrocytes post-operatively, but no improvement in a further case. Loeb, Moore and Dubach (1953) described a patient who no longer required transfusions after splenectomy. Green and his co-workers (1953) also referred to a single patient who was benefited by splenectomy. Similar results were reported by Cartwright and others (1955) and Korst, Clatanoff and Schilling (1956).

More recently, Bouroncle and Doan (1962), in a review of 110 patients, stated that 24 of them had undergone splenectomy (for hæmolysis or thrombocytopenic purpura) and that 14 of them did well, nine no longer requiring transfusions after operation.

Less satisfactory results have been reported by other authors. For example, Richmond and Duncan (1956) reported that hæmolysis was arrested in one of three patients, and Hutt, Pinniger and Wetherley-Mein (1958), in a review of 21 cases, reported that only two of them who had progressive hæmolytic anæmia improved significantly. Pitcock and co-workers (1962) stated that two patients benefited out of nine, while Strumia and his co-workers (1962), in a review of the results of 29 splenectomies, reported that benefit from the operation was less regular in myelosclerosis (and in chronic granulocytic leukæmia) than in chronic lymphocytic leukæmia.

Six patients studied by the author and his colleagues have recently been submitted to splenectomy. In nearly all of them the reason for the operation was an ever-increasing blood transfusion requirement. The results have been disappointing and the post-operative mortality and morbidity have been high. In only two of these patients was the operation of benefit. One of these patients has been described as Case 21 (p. 762); the other derived hæmatological benefit from the operation but persistent pulmonary infection caused his death 4 months later. The remaining four patients all died for various reasons (mostly of infection or thrombo-embolism) within the immediate post-operative period.

The present author thinks that splenectomy has a place in the treatment of myelosclerosis but that too much should not be expected of it; its limitations and dangers must be recognized. The role and importance of the spleen as an erythropoietic organ have probably been overrated; in patients with marked hæmolysis the hæmolytic function of the spleen is probably the more important. Each case in which splenectomy is contemplated should be carefully investigated, and studies carried out with ^{51}Cr and ^{59}Fe; if it is found that excess hæmolysis is an important factor in the patient's anæmia and that some at least of the excess hæmolysis is taking place in the spleen, then a good case for splenectomy can be made out, particularly if the patient already needs transfusions to sustain him. What must not be done is to postpone the operation to the last possible moment when the patient is in the terminal stage of his disease. If this is done, then the operative mortality will be unacceptably high and the chances of a sustained and worthwhile improvement slender.

HÆMOLYTIC ANÆMIA IN ASSOCIATION WITH CARCINOMATOSIS

Anæmia is a common complication of cancer; and where the disease is widespread it is almost always present and may be severe. The anæmia has a complicated origin: blood loss by external hæmorrhage or hæmorrhage into, or in the vicinity of, the tumour, interference with erythropoiesis by metastatic invasion of the bone marrow, the effects of concurrent sepsis, and hæmolysis—all may play a part in its causation. These factors not unnaturally vary in importance from patient to patient. In some, excessive hæmolysis appears to be the dominant mechanism; occasionally, indeed, patients present first with all the signs pointing to a "primary" hæmolytic anæmia, and it is only later in the course of the disease that an underlying tumour is discovered.

Overt Hæmolytic Anæmia

There are by now many descriptions of severe anæmia, clearly predominantly hæmolytic in origin, associated with disseminated carcinoma. Usually the bone marrow has been widely infiltrated.

Early reports of typical cases are to be found in the papers of Waugh (1936), Caroli and Lavergne (1937), Lucey (1939), Holmes and McCall (1940), Davis (1944), Stats, Rosenthal and Wasserman (1947), Frandsen (1949), Hogeman (1953), Veflingstad (1953), Dacie (1954, Cases 22 and 23), Cotti (1954), Frumin, Mendell and Meranze (1954), Giraud, Latour and co-workers (1955), Sohier, Juranies and Aub (1957) and Capelli, Cioni and Giannardi (1957–8). In these authors' patients the tumours originated in the prostate, breast, stomach, lung, pancreas, colon and ovary, respectively. The early literature was reviewed by Paraf and Dausset (1952) and by Wasserman and his colleagues (1955). Paraf and Dausset stated that the stomach is the commonest site for the primary tumour. More recent reviews include those of Hyman (1963) and Landow (1964) and other reports include a case discussed at the Massachusetts General Hospital (Case 571–1962) and patients described by Zittoun and co-workers (1965), Brook and Konwaler (1965) and Stratford and Tanaka (1965).

In some instances a rather rapid onset of severe anæmia, perhaps with pyrexia, was the first sign of the patient's illness; in other patients the anæmia was the first sign that the disease had become disseminated following previous excision of the primary tumour. The recent case report of Dumont and co-workers (1965) can be quoted as an example of acute severe hæmolytic anæmia (in a young woman) the cause for which (widely disseminated carcinoma of the stomach) was only found *post mortem*.

More usually, severe anæmia with signs of hæmolysis occurs as a terminal event as part of a rapid deterioration in the general condition of the patient (the tumour's host) (Sohier, Juranies and Aub, 1957).

Laboratory Findings. Anæmia may be severe, with the hæmoglobin concentration falling to 4 g. per 100 ml. or less and the erythrocyte count to 1·0 million per cu. mm. or less. The reticulocyte count usually ranges between 5% and 20% but may be much higher. The MCV lies within 90 cu.μ and 120 cu.μ as a rule. The total leucocyte count varies: usually it is raised; it may even exceed 30,000 per cu. mm. The majority of the cells are neutrophils; but almost always 1–10% of myelocytes are present, and occasionally a few promyelocytes or even myeloblasts. There is usually a moderate degree of thrombocytopenia, and occasionally this may be severe and lead to a hæmorrhagic diathesis (Frandsen, 1949).

The erythrocytes characteristically vary considerably in size and shape and there is usually a moderate to marked degree of polychromasia and/or punctate basophilia. Normoblasts are almost invariably present, in addition to immature granulocytes—hence the term "leuco-erythroblastic anæmia" (Vaughan, 1936). Rounded spherocytes may be seen in some cases; in others, a variable number of irregularly contracted and distorted erythrocytes or cell fragments are present (Dacie, 1954, p. 346 (Cases 22 and 23); Cotti, 1954; Brain, Dacie and Hourihane, 1962; *Case Records of the Massachusetts General Hospital*, 1962; Landaw, 1964; Dumont *et al.*, 1965; Stratford and Tanaka, 1965; *Clinicopathologic Conference*, 1965; Zittoun *et al.*, 1965; Brook and Konwaler, 1965; Brain, Dacie and Azzopardi, 1966) (Figs. 199–203).

Erythrocyte osmotic fragility is often increased (Waugh, 1936; Caroli and Lavergne, 1937; Lucey, 1939; Stats, Rosenthal and Wasserman, 1946, etc.). The plasma-bilirubin concentration is usually moderately raised but it seldom exceeds 2 mg. per 100 ml. unless tumour metastases are present in the liver or obstruct the bile ducts.

Serological Findings

Tests for abnormal auto-antibodies are almost always negative in the hæmolytic anæmia associated with carcinomatosis. However, the direct antiglobulin test has occasionally been reported to have been positive, *e.g.*, by Jordan and Dingle (1949), Cotti (1954), Frumin, Mendell and Meranze (1954), Renfer and Hässig (1955) (who obtained four clear positives and four weak positives out of a total of 67 patients tested), Giraud and his co-workers (1955), West, Ley and Pearson (1955), Johnston (1956), Sohier, Juranies and Aub (1957), Jenkins and Marsh (1961) and Ellis and Westerman (1965). In many of the above-quoted cases the antiglobulin reaction was a weak one.

The history of Giraud and his co-workers' (1955) patient was remarkable in that she presented as a case of auto-immune hæmolytic anæmia with no signs of malignancy. Splenectomy was eventually carried out but the anæmia persisted. Six months later deep jaundice supervened and a carcinoma of the pancreas was found *post mortem*. The case report published by Boulet and his co-workers (1956) was equally remarkable. This patient developed hæmolytic anæmia with a positive antiglobulin test the day after hypophysectomy for duodeno-pancreatic carcinoma. It was suggested that the hæmolysis may have been precipitated by a sudden withdrawal of ACTH.

Jenkins and Marsh's (1961) patient had cystadenocarcinoma of the ovaries and later developed a carcinoma of the breast. She had been treated by oophorhysterectomy, X-radiation and transfusions. Two months after the last transfusion she was found to have a hæmoglobin of 4·5 g. per 100 ml. and 10% reticulocytes. The direct antiglobulin test was positive, the reaction being of the non-γ type, and the serum contained an antibody which agglutinated stored erythrocytes.

In only one out of 59 cases of secondary auto-immune hæmolytic anæmia investigated by the author over a 15-year period did a disseminated carcinoma appear likely to be the underlying cause (see p. 343, Volume II). One further case has been investigated more recently. This patient had a carcinoid tumour of the ampulla of Vater and gave a clearly positive direct antiglobulin test of the non-γ type. His serum contained two antibodies—an anti-I cold agglutinin of moderately high titre and thermal amplitude and a "non-specific" hæmolysin active against enzyme-treated erythrocytes at 37° C.

In the author's experience antiglobulin tests carried out on patients suffering from various types of carcinoma (with or without anæmia) have rarely been positive. (This is admittedly contrary to other workers' experience (see p. 789).) For instance, a routine recent screening of 50 consecutive carcinoma patients gave only two weak positive tests, using the laboratory's standard potent "wide-spectrum" antiglobulin serum at dilutions of 1 in 4 to 1 in 256. The reactions in each case were of the non-γ type and the incidence (4%) was certainly not greater than the incidence of similar results in the general hospital population—8% positive out of 489 patients (Worlledge, unpublished observations).

The case histories of three personally studied patients who had severe hæmolytic anæmia accompanying disseminated carcinoma are described below (Cases 22 to 24).

Case Report: Carcinoma of Stomach Presenting with Severe Hæmolytic Anæmia and Thrombocytopenia

Case 22. The patient, K. L., was a married woman aged 69. She was admitted into hospital giving a short history of loin pain, blood in the urine and easy bruising. Pigmented cutaneous warts had appeared within the last 6 weeks. She stated that she had had a "dragging" pain in the stomach for the last 2 years or so. She was found to be anæmic and was transfused and later transferred to Hammersmith Hospital for further investigation.

Physical Examination. She was a pale obese woman; many subcutaneous bruises were present and she had pigmented papillomata in the axillæ and groins (acanthosis nigricans). There was some tenderness in the left hypochondrium; the spleen could just be felt.

Laboratory Findings. On admission the hæmoglobin was 3·9 g. per 100 ml., erythrocyte count 1,700,000 per cu. mm., PCV 14·5%, and reticulocyte count 10%. The leucocyte count was 6,000 per cu. mm., with 74% neutrophils, 12% metamyelocytes and 2% myelocytes. The platelet count was 19,000 per cu. mm. A small number of erythroblasts were present (4 per 100 leucocytes). Stained films showed moderate anisocytosis, poikilocytosis and polychromasia as well as microspherocytes and other contracted and distorted cells and some cell fragments (Fig. 200).

The serum bilirubin was 1·8 mg. per 100 ml. and alkaline phosphatase 31 K.A. units. The blood urea was 53 mg. per 100 ml. The electrophoretic strip of serum proteins appeared normal. The serum iron was 230 μg. per 100 ml., and tests for occult blood in fæces were positive. The direct antiglobulin test was negative.

Bone-marrow puncture yielded few marrow cells. However, two small clumps of unidentified darkly-staining cells were seen and these were thought probably to be malignant cells.

Radiographs of the stomach showed appearances suspicious of an infiltrative lesion of the lesser curve and quite marked mucosal atrophy.

A diagnosis of microangiopathic anæmia was made, probably secondary to a carcinoma of the stomach.

Further Progress. The patient's general condition deteriorated and she died on the 8th April 1961 despite blood transfusions.

Post-mortem examination showed a carcinoma of the pylorus of the stomach with metastases in abdominal lymph nodes and in the sternum and bodies of the vertebræ. The spleen weighed 578 g. Sections showed that the tumour was of the mucin-secreting signet-ring type. The vertebral marrow was extensively infiltrated by carcinoma. The spleen and liver contained a great excess of iron, although apparently free from tumour. Small thrombi were seen in some of the arterioles in the heart and lungs.

Summary. A case of carcinoma of the stomach of the mucin-secreting signet-ring type, with extensive metastases in lymph nodes and bones, complicated by a severe anæmia of the microangiopathic hæmolytic type.

Case Report: Adenocarcinoma (Primary Site Unknown) Presenting as Acquired Hæmolytic Anæmia

Case 23. R. D., a male aged 55, was admitted into Poole General Hospital with a 3 weeks' history of anorexia and lumbo-sacral pain and increasing pallor and shortness of breath. He was found to have a severe hæmolytic anæmia and was transfused with 6 l. of blood within a 3-week period. He was then transferred to Hammersmith Hospital for further investigation.

Physical Examination. He was found to be a pale, slightly jaundiced man. The spleen could just be felt, but otherwise there were no abnormal physical signs; in particular, the liver and peripheral lymph nodes were not enlarged. The urine contained an excess of urobilinogen but no bile. There was pyrexia up to 100–101° F. daily.

Laboratory Findings. On admission, the hæmoglobin was 10·5 g. per 100 ml., PCV 31%, reticulocyte count 12%, leucocyte count 7,000 per cu. mm., with 58% neutrophils and 2% myelocytes. The platelet count was 22,000 per cu. mm. The hæmoglobun fell within the next 5 days to 7·5 g. per 100 ml. and the leucocyte count fell also, the minimum figure being 1,800 per cu. mm.

Stained films showed moderate anisocytosis and poikilocytosis and polychromasia with, in addition, spherocytes, distorted cells and cell fragments (Fig. 201). A small number of erythroblasts (3–9 per 100 leucocytes) were constantly present.

Erythrocyte osmotic fragility was increased: lysis commenced at 0·55% NaCl and the MCF was 0·485% NaCl. The direct antiglobulin test was negative.

The serum bilirubin was 0·7 mg. per 100 ml.; alkaline phosphatase was high (76·5 units); acid formol-stable phosphatase 2 units; serum albumin 2·3 g. and serum globulins 3·5 g. per 100 ml. The blood urea was 28 mg. per 100 ml. Tests for occult blood in fæces were positive. The urine was free from albumin, Bence-Jones protein or hæmosiderin.

Bone-marrow puncture was attempted twice: on the first attempt (in the iliac crest) no marrow was obtained; on the second attempt (manubrium sterni) a few cellular marrow fragments were aspirated. Films showed erythroblastic hyperplasia, but in addition there were a few clusters of abnormal cells with eccentric nuclei containing PAS-positive material in their cytoplasm.

A diagnosis of carcinomatosis of the bone marrow with secondary microangiopathic hæmolytic anæmia was made. The patient's erythrocytes (a mixture of his own and transfused blood) were labelled with ^{51}Cr: the ^{51}Cr T$\frac{1}{2}$ was 7 days.

Radiographs showed partial collapse of the bodies of two dorsal vertebræ and probable areas of bone destruction in the pelvis.

Treatment and Progress. Only symptomatic treatment appeared possible; cortisone at a dose of 300 mg. daily did not influence the progression of the anæmia and he received 5·5 l. of blood over a period of 19 days before being transferred back to Poole General Hospital where he died a few days later.

A necropsy was carried out by Dr. W. McNaughton. This showed disseminated carcinoma with secondaries in retroperitoneal lymph nodes and bones, but no primary tumour could be identified. The spleen weighed 670 g. The immediate cause of death was pulmonary œdema and bronchopneumonia.

Sections of lymph nodes showed extensive infiltration with adenocarcinoma and many veins and lymph channels were invaded by carcinoma cells. The spleen contained numerous foci of extramedullary hæmopoiesis and an excess of iron.

Summary. A case of widely disseminated adenocarcinoma complicated by a severe anæmia of microangiopathic hæmolytic type.

Case Report: Adenocarcinoma of Lung, with Hæmolytic Anæmia and Extensive Venous Thrombosis

Case 24. A male, C. W., aged 68, was admitted to Hammersmith Hospital on the 19th July 1956, complaining of a pain in the chest for 7 weeks, and painful swelling of the right leg for 2 weeks and of the left for the last 4 days. He had also become increasingly breathless and had developed a cough associated with mucoid sputum; he had lost 15 kg. in weight since his illness started.

Physical Examination. He was a pale, breathless and cyanosed man. Rhonchi and rales were present throughout the lung fields. The liver was enlarged 5 cm. below the right costal margin but the spleen could not be felt. A small left supraclavicular node was palpable. Thrombosed tender veins could be palpated in both calves.

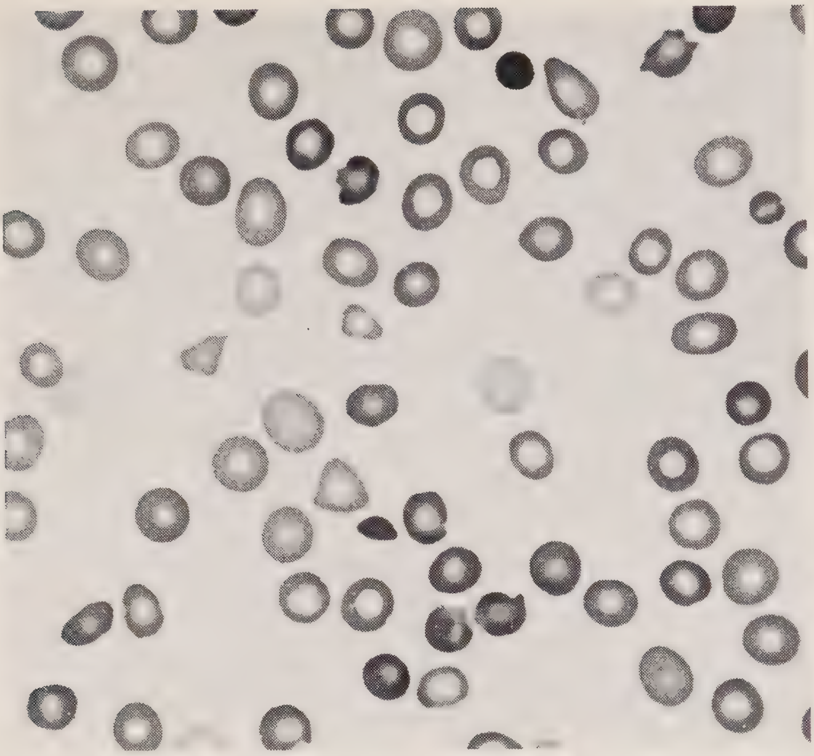

FIG. 200. Photomicrograph of a blood film of a patient suffering from disseminated carcinoma of the stomach (Case 22). There is a moderate degree of anisocytosis and poikilocytosis, and a few spherocytes and a moderate number of irregularly contracted cells are also present. ×700.

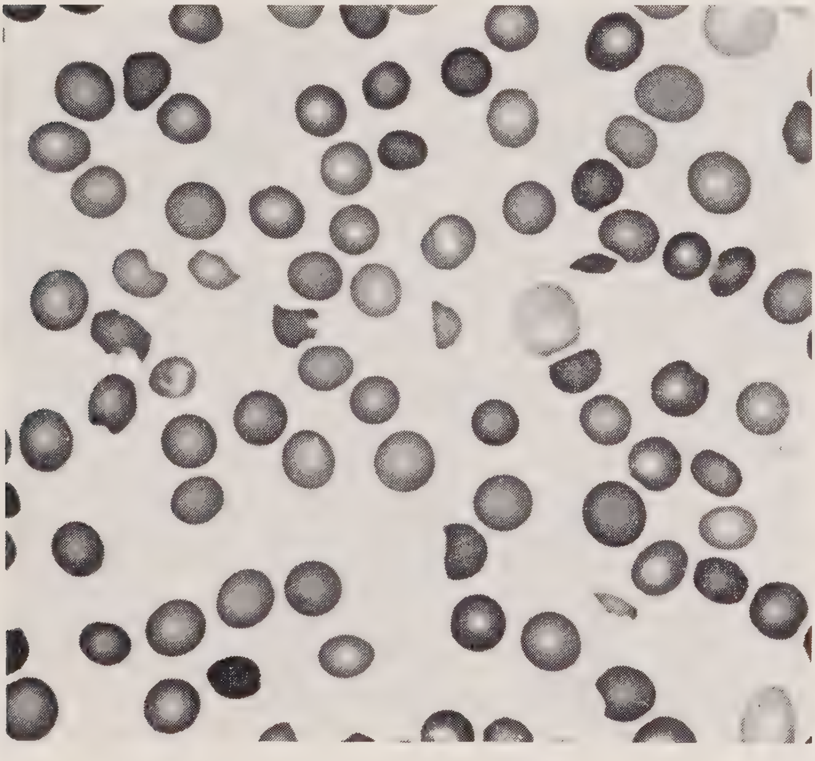

FIG. 201. Photomicrograph of a blood film of a patient suffering from a disseminated carcinoma (Case 23). There is a moderate degree of anisocytosis, poikilocytosis and spherocytosis, and quite large numbers of irregularly contracted and distorted cells and some cell fragments are present. ×700.

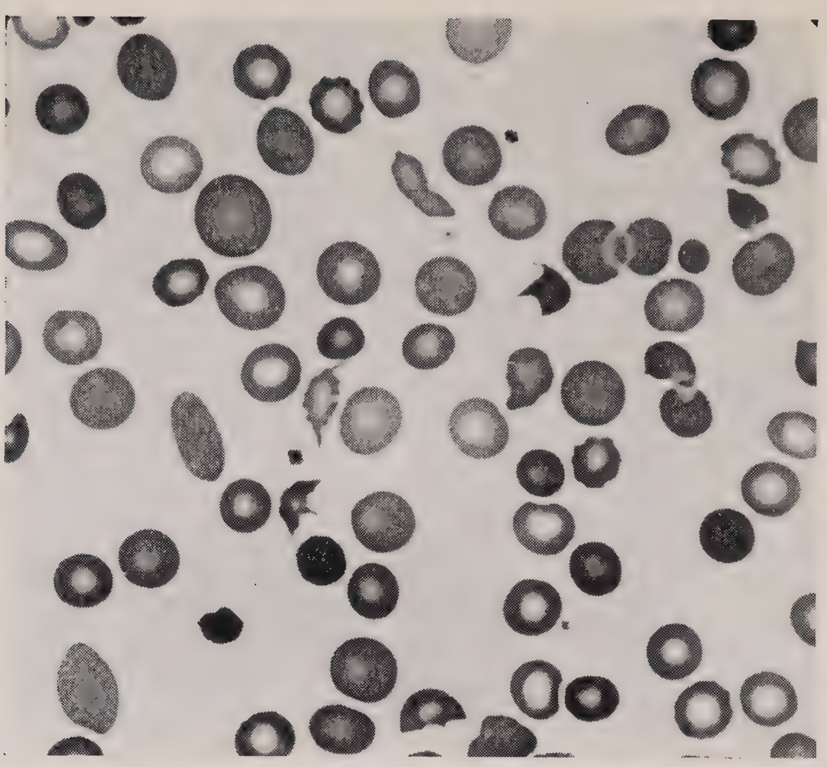

FIG. 202. Photomicrograph of a blood film of a patient suffering from disseminated carcinoma of the lung (Case 24). There is marked anisocytosis, macrocytosis and anisochromasia. Some microspherocytes are present and also quite large numbers of irregularly contracted and distorted cells. × 700.

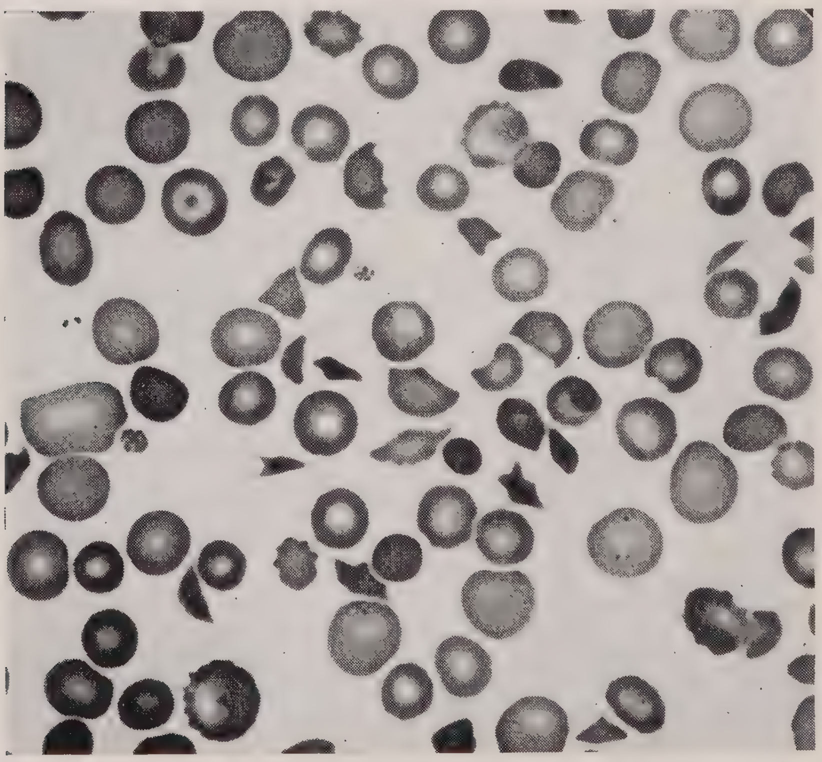

FIG. 203. Photomicrograph of a blood film of a patient suffering from disseminated carcinoma of the breast. There is marked anisocytosis and macrocytosis, and numerous contracted and distorted cells and cell fragments are present. This film represents the most florid "microangiopathic" blood picture yet seen by the author in carcinomatosis. × 700.

The urine contained protein, and erythrocytes and granular casts were present in the urine deposit.

Laboratory Findings. The hæmoglobin was 7·6 g. per 100 ml., erythrocyte count 2,700,000 per cu. mm., with 22% reticulocytes; the leucocyte count was 23,000 per cu. mm., with 83% neutrophils, and platelet count 144,000 per cu. mm. Stained films showed occasional myelocytes and erythroblasts. There was marked anisocytosis and poikilocytosis, and macrocytes, spherocytes and quite frequent contracted and distorted cells and cell fragments were also present (Fig. 202).

Bone-marrow aspiration showed a cellular marrow in which normoblastic erythropoiesis and leucopoiesis were both active. No malignant cells were seen.

The direct antiglobulin test was weakly positive; the blood urea was 47 mg. per 100 ml., serum albumin 1·7 g. per 100 ml. and serum globulins 4·5 g. per 100 ml.

The urine contained a small amount of protein and urobilin but no bile pigment. Later heavy proteinuria developed.

Course and Treatment. The patient was given antibiotics and his pyrexia subsided. Dindevan therapy was started but the thromboses in his legs extended, nevertheless, and his legs became increasingly œdematous. Transfusions were only of temporary benefit. A few days before death many of the superficial veins, except the external jugulars, appeared to be thrombosed, and both feet became cold and blue suggesting incipient gangrene. He died on the 21st August 1956.

Post-mortem examination showed a carcinoma of the lung, with extensive lymphatic permeation of both lungs and secondaries in the stomach and liver (1,780 g.). The spleen was small (80 g.) and was free from tumour. Secondaries were, however, also found in the hilar nodes of each lung and in para-aortic nodes and in the sternal, vertebral and iliac marrow. The right renal pelvis contained a papillary transitional-cell tumour. The main veins of both legs were filled with thrombi and on the right side the thrombi extended into the pelvis. Thrombi were seen, too, in small arm veins. Emboli (thrombi) were present in branches of the pulmonary artery in the lower lobes of both lungs.

Sections of the tumour showed it to be a mucin-secreting adenocarcinoma of bizarre cytology. The spleen contained an excess of iron but was free from tumour. Many thrombi, both recent and organized, were present in veins in the muscles and in the skin of the lower limbs. An endothelialized thrombus was present in a small arteriole in the heart.

Summary. A case of adenocarcinoma of the lung, with extensive dissemination to lymph nodes, liver and bone marrow. Extensive thromboses in veins and an anæmia of microangiopathic hæmolytic type developed as a complication.

Incidence of Hæmolytic Anæmia in Carcinomatosis: Latent Hæmolytic Anæmia

There have been a number of surveys published in recent years on the incidence of excessive hæmolysis in carcinomatosis; and in some of these the frequency of overt hæmolysis has been compared with the frequency of latent hæmolysis demonstrated by means of erythrocyte survival studies.

The incidence of *overt* hæmolytic anæmia in Shen and Homburger's (1951) series of 116 anæmic patients with advanced cancer was small (2·6%). Their criteria for the diagnosis of hæmolytic anæmia were "either a positive Coombs test or an increase in the osmotic or mechanical fragility of the red cells together with reticulocytosis, hyperbilirubinemia and an increased output of blood pigments." A smaller series of patients with disseminated carcinoma of the breast was studied by West, Ley and Pearson (1955): clear evidence of hæmolysis was found in only one patient out of the 16 patients who were anæmic.

As might have been anticipated, erythrocyte survival studies have shown that the incidence of hæmolysis is much greater than the above-mentioned data suggest. Brown (1950) studied five cases of carcinomatosis and, using the Ashby method, obtained mean cell life-spans of 16–53 days. Sheets, Hamilton, DeGowin and Janney (1954) transfused normal blood to four patients with carcinoma and found that it was eliminated unusually rapidly and in a random manner. In three other patients, suffering from carcinoma of the cervix, the rate of hæmolysis was observed to increase 7–10 days after commencing X-ray or radium treatment.

Hyman (1954), Hyman and Harvey (1955) and Hyman, Gellhorn and Harvey (1956) compared the incidence of hæmolysis as indicated by conventional methods (urobilinogen excretion figures, reticulocyte counts, etc.) and that demonstrated by cell-survival studies. Only in a small minority of cases did their routine tests provide evidence for a moderate increase in hæmolysis whereas almost all the survival experiments demonstrated a diminution in the life-span of transfused normal erythrocytes. Hyman and Harvey (1955), in repeated studies on two patients, demonstrated a further impairment in survival paralleling a worsening of the patients' disease. Hyman, Gellhorn and Harvey (1956) performed the reverse experiment of transfusing patients' blood to normal volunteers: in most instances a normal or nearly normal life-span was observed and it was concluded that the most important factor in the hæmolysis was an extracorpuscular one.

Johnston (1956) studied 10 patients, using ^{51}Cr, and obtained evidence for a decreased erythrocyte life-span in three of them. He concluded that hæmolysis was probably a common event in the terminal stages of the disease. Miller, Chodos, Emerson and Ross (1956), using mainly the Ashby method, demonstrated increased hæmolysis in five out of 13 patients. Chodos, Miller, Ross and Emerson (1956) also reported data on fæcal urobilinogen excretion. Although the total daily excretion was within the normal range in all 15 patients studied, when the hæmolytic index was calculated (see p. 9, Part I), this was found to be above normal in four patients and high normal in two.

Hyman (1958) studied 51 patients, all with metastases in bone. Routine studies provided evidence of slight hæmolysis in 14 out of 30 patients and of moderate hæmolysis in one patient. Mild to moderate hæmolysis was demonstrated by the Ashby and ^{51}Cr methods in 26 out of 34 patients. In several patients an improvement in cell survival was demonstrated after X-ray or hormonal therapy, or adrenalectomy.

Patients' blood transfused to volunteers survived normally in 11 out of 18 instances.

Ultmann (1958) carried out ^{51}Cr studies, including surface counting, and obtained evidence for splenic sequestration in patients giving negative antiglobulin tests. This was so in three out of four patients with disseminated carcinoma; he also showed that radioactivity accumulated in a large metastasis from a hypernephroma.

Jandl (1959), in discussing the pathogenesis of a severe terminal hæmolytic anæmia in a patient with adenocarcinoma of the breast, reported that normal cells were destroyed as fast as the patient's own cells. ^{51}Cr studies suggested that in this particular case the liver was a more important hæmolytic organ than the spleen; the direct antiglobulin reaction was negative and methæmalbumin was demonstrated in the serum.

HÆMOLYTIC ANÆMIA ASSOCIATED WITH OVARIAN TUMOURS

There are a few reports of hæmolytic anæmia developing in association with ovarian tumours, usually dermoid cysts or teratomata. Because of the remarkable nature of this anæmia and because hæmolysis characteristically ceases when the tumour is removed, this rare association will be discussed in some detail.

West-Watson and Young (1938) described the history of a patient aged 44 years who developed a severe hæmolytic anæmia with marked spherocytosis. Splenectomy was performed but the hæmolysis continued unabated. Four months later laparotomy was undertaken to exclude the presence of splenunculi. Instead an ovarian teratoma was discovered; this was removed and the patient made an uninterrupted recovery. Watson (1939) described the occurrence of hæmolytic anæmia in a young woman aged 19, which was associated with a left ovarian mass. This proved at laparotomy to be a large cyst containing fluid; its removal had no effect on hæmolysis, but splenectomy carried out later was followed by slow improvement and eventually recovery.

Singer and Dameshek (1941) recorded an almost exactly similar case to that of West-Watson and Young. Their patient, a woman aged 47 years, had a severe hæmolytic anæmia with microspherocytosis of about 5 months' duration. Splenectomy resulted in some improvement but the patient still required to be transfused post-operatively. Eventually, 7 months after splenectomy, a dermoid cyst of the ovary was found at laparotomy and removed. The hæmolytic process then subsided and within 4 months the blood picture became normal.

Since these early reports other accounts of a similar nature have appeared in the world literature (Jones and Tillman, 1945; Lindeboom, 1950; Allibone and Collins, 1951; Wuhrmann, 1954; André, Dreyfus and Salmon, 1955; Procházka, 1956; Barry and Crosby, 1957; Miescher, von Rechenberg, Berger and Holländer, 1958; Müller and Schubothe, 1958 and Müller, Schubothe and Elert, 1958; Heeres, 1960; Szirmai, 1961; Larrain, Del Solar and Vargas Molinare, 1963, and McAndrew, 1964). Barry and Crosby's and Müller, Schubothe and Elert's accounts include useful reviews of the literature.

Jones and Tillman's (1945) patient differed from most of the others in that the ovarian tumour proved to be a pseudo-mucinous cystadenocarcinoma. Following its removal, however, the hæmolysis subsided and splenectomy was not carried out.

Allibone and Collins's (1951) report was also unusual. Their patient was a 4-year-old girl who presented with a severe spherocytic anæmia of about 3 months' duration. An abdominal tumour was found which after removal was found to be a cystic teratoma of the ovary. The child's blood picture rapidly returned to normal after operation. Her spleen was not removed.

Miescher, von Rechenberg, Berger and Holländer's (1958) patient was also unusual in that she was suffering from tuberculous endometritis and had an ovarian tumour which was apparently part fibroma and part myofibroma. Heeres's (1960) patient was reported as having bilateral systic ovarian tumours.

What is particularly remarkable about the above series of patients is that all the patients (if post-operative deaths are excluded), except that of Watson (1939), responded dramatically to removal of their ovarian tumour or tumours, irrespective apparently of the exact nature of their tumour. Most of them were suffering from severe hæmolysis with marked microspherocytosis and had high reticulocyte counts. Steroid therapy or splenectomy was generally ineffectual.

Serological Findings. The direct antiglobulin test has been found to be positive in all the cases published in recent years, with the exception of those of Allibone and Collins (1951) and McAndrew (1964), and there seems little reason to doubt but that the peculiar hæmolytic anæmia which may occasionally accompany ovarian tumours is auto-antibody induced. It is of particular significance that in each case the antiglobulin test became negative following removal of the ovarian tumour.

The nature of the abnormal antibody apparently varies—as it does in "idiopathic" cases. In André, Dreyfus and Salmon's (1955) case, the direct antiglobulin test was but doubtfully positive, but auto-agglutination took place in albumin and when the patient's erythrocytes were enzyme-treated. The tests became negative after ovariectomy. In the patient of Miescher and his co-workers (1958) the direct antiglobulin reaction was of the γ-globulin type and free unidentified antibody was present in the patient's serum. This antibody was not adsorbed by the tumour tissue, and no auto-antibody could be demonstrated fixed to it. Müller, Schubothe and Elert (1958) demonstrated hæmolysis and erythrophagocytosis when they carried out the Ehrlich finger test (p. 359, Part II).

Auto-Immune Hæmolytic Anæmia Associated with Cysts not of Ovarian Origin

In a small number of cases there seems to have been a possible causal relationship between an intra-abdominal cyst and hæmolysis.

A possible early example was the patient of Suarez and Etcheverry (1936–7) who at laparotomy for a long-continued severe hæmolytic anæmia was found to have a large dermoid cyst attached to the spleen. Sandøe's (1953) case is more convincing. This patient was a 30-year-old woman who had a hæmolytic anæmia and gave a positive antiglobulin test. When a mesenteric cyst was removed all signs of hæmolysis disappeared and the direct antiglobulin test became negative. Ten months later the antiglobulin test was positive again although there were no signs of hæmolytic anæmia. The cyst contained an irregular antibody at high titre, and Ahrengot (1953) considered that the cyst could have been the source of the antibody (see p. 598, Part II).

Hæmolytic Anæmia in Sarcoma in Man

A few examples of hæmolytic anæmia in patients suffering from sarcoma have been reported. This is particularly true of Kaposi's sarcoma (Hogeman, 1953; Martensson and Henrikson, 1954, and Zemek, Strom, Gordon and Elguezabel, 1964). The direct antiglobulin test was negative in Hogeman's patient, whose syndrome seemed to resemble hypersplenism, but positive in that of Martensson and Henrikson. The patient of Zemek and his colleagues was particularly interesting in that her hæmolytic anæmia, with a positive antiglobulin test, had preceded by 8 years the signs of the sarcoma.

Norcross (1963) described a patient with a fibrosarcoma of the back whose anæmia paralleled in severity the growth of the tumour.

Hæmolytic Anæmia in Laboratory Animals with Experimental Tumours

The anæmia which accompanies the growth of tumours experimentally produced in laboratory animals has been quite extensively studied, and as in man excess hæmolysis has been shown to play a part in its causation. Only brief reference to some recent work in this field which has a bearing on human hæmolytic mechanisms can be given.

A great deal of work has been carried out with hamsters bearing a variety of tumours, mainly sarcomas or melanoma. Sherman (1959, 1962) has stressed, in particular, the importance of tumour necrosis in relation to the development of anæmia, while Betts, Rigby, Emerson and Friedell (1961) reported the frequent finding of positive antiglobulin tests. Rigby, Betts, Friedell and Emerson (1962) have stressed the importance of reticulo-endothelial cell overactivity in the animals in relation to erythrocyte destruction and Price and Greenfield (1958) and Price, Greenfield, Sterling and MacCardle (1959) the role of hæmorrhage into the tumour, followed by extravascular destruction of the extraversated erythrocytes.

There is also a considerable literature on the occurrence of a hæmolytic anæmia in tumour-bearing rats which appears to be due to a transmissible sub-cellular agent, probably a virus (Sacks and Egdahl, 1960; Belcher and Simpson, 1960; Simpson and Belcher, 1962). In mice, too, Rask-Nielsen (1964) has reported the development of Coombs-positive hæmolytic anæmia, associated with generalized amyloidosis, following the transmission of sub-cellular leukæmic material.

The relevance of these important observations and concepts to the pathogenesis of the hæmolytic anæmias associated with malignant disease in man is discussed on p. 790.

Treatment of Hæmolytic Anæmia in Association with Carcinomatosis, etc.

Treatment of anæmia in disseminated carcinoma or sarcoma can as a rule only be symptomatic, with transfusion of blood being often the best that can be done. In relatively early cases of dissemination any arrest or regression of the tumour following chemotherapy or irradiation will in most instances be paralleled by improvement in the patient's anæmia and in a lessening of the hæmolysis. In the case of large local tumours anæmia may regress strikingly if the local tumour can be effectively treated, as for example in Norcross's (1963) patient with a fibrosarcoma.

PATHOGENESIS OF INCREASED HÆMOLYSIS IN CARCINOMATOSIS, ETC.

There is no question but that the anæmia of disseminated carcinoma and other tumours has a complicated origin. This discussion will be confined to the problem of excess hæmolysis and how this is brought about. Possible mechanisms are: the effects of auto-antibodies; loss of blood locally into and around the tumour; stimulation of phagocytic reticulo-endothelial cells by metabolic products of tumour growth; damage to erythrocytes by intimate contact with tumour tissue; the presence of tumour cells in bone marrow leading to the production of abnormally short-lived erythrocytes, and the effects of viruses.

Auto-Antibodies. Overt auto-immune hæmolytic anæmia is clearly a rare accompaniment of local or disseminated carcinoma in man. However, the possibility that the coating of erythrocytes with globulin contributes to their early destruction, in some cases at least, cannot altogether be dismissed. Antiglobulin tests have been reported to be positive in a relatively large proportion of patients by some workers, both in man and in experimental animals (see below). The reported reactions have, however, mostly been weak ones, demonstrable only in strong concentrations of antiglobulin serum, and it should perhaps be added that in the author's laboratory, at least, the results of antiglobulin tests using routine, but potent, antiglobulin sera in a variety of dilutions do not suggest that cancer subjects give positive reactions under these conditions at all frequently (see p. 780). It would be fair to say, too, that, even if it could be indubitably shown that the erythrocytes of cancer patients were in fact often coated with normal globulins in abnormal amounts or with abnormal globulins, it could not be assumed, without further evidence which might be very difficult to obtain, that such coating led to increased hæmolysis.

In man, Green, Wakefield and Littlewood (1957) reported obtaining positive direct antiglobulin tests, using an antiglobulin serum diluted 1 in 10, in 43% of patients with cancer and 15% of patients without cancer. The difference between the cancer patients and the controls was considered to be significant even if the results in patients with tumours of the reticulo-endothelial system, in which the percentage of positive results was the highest, were excluded. The "antibody" coating the erythrocytes was thought to be a non-gamma-globulin lipoprotein complex. Green and his colleagues concluded that anæmia in cancer might commonly be auto-immune in origin; they suggested that the tumour not the host was the source of the antibody, with the tumour cells reacting against tissue specific antigens in the host, which the tumour cells themselves had lost. Davis, Green and Tymms (1961) later reported that electronmicrographic studies indicated that the surface structure of erythrocytes in cancer patients was frequently abnormal, a finding which they interpreted as supporting their view that the cells were coated by incomplete antibody.

Adelsberger and Zimmerman (1959) also concluded that the erythrocytes of cancer patients differed from those of healthy subjects. By immunizing rabbits with the two types of cells, it appeared as if the erythrocytes of cancer patients had an altered antigenicity. The hæmagglutination reactions were similar to those obtained when rabbits were immunized with trypsinized normal cells.

Betts, Rigby, Friedell and Emerson (1962), like Green and his colleagues (see above), obtained positive direct antiglobulin reactions in cancer patients. Using an antiserum prepared in rabbits by the injection of whole human serum and Freund's adjuvant, positive results were obtained in 48 of 52 cancer patients (the titres were low, 16–64), while in 104 random controls only four tests were positive. Significantly, however, positive tests were also obtained in 20 of 42 patients with active tuberculosis and in individual patients with rheumatoid arthritis or systemic lupus erythematosus; and positive results were obtained, too, in 13 of 46 pregnant women and in 10 out of 35 post-partum women. Betts and his colleagues considered that an abnormal globulin was being produced and that this was perhaps derived from the tumour or from necrotic tumour tissue; they concluded that the globulin might play a part in bringing about premature hæmolysis of a cell coated by it.

The occurrence of positive antiglobulin tests in tuberculosis and in pregnancy as well as in the cancer cases suggests that tissue destruction or involution or inflammation provide the background for the formation or liberation of the globulin(s). The raised sedimentation rate in tumour cases, tuberculosis and pregnancy, no doubt has a similar origin.

Further evidence that the erythrocytes of cancer patients might be abnormal has received support from the work of Adelsberger and Becker (1965) who found that cancer patients' erythrocytes were more easily lysed by cancer tissue supernatant than were normal cells.

In tumour-bearing hamsters, too, antiglobulin tests are frequently positive. Betts, Rigby, Emerson and Friedell (1961) obtained positive results in 12 out of 15 animals when an antiglobulin serum which had not been heat-inactivated was used (Freund's adjuvant had been used in the making of the antiglobulin serum). They suggested that the occurrence of positive tests preceded the development of anæmia, and Rigby, Betts, Friedell and Emerson (1962) concluded that destruction by a hypertrophied reticulo-endothelial cell system of the globulin-coated erythrocytes was an important mechanism in the pathogenesis of the anæmia of malignancy.

Role of Tumour Mass and Tumour Necrosis. In most cases of human cancer in which marked hæmolytic anæmia has been present the carcinoma has been widely disseminated, and it is reasonable to suppose that other things being equal, the greater the volume of tumour tissue the greater its systemic effects will be. Judging from animal work the occurrence of necrosis and the amount of necrosis are particularly important.

Sherman (1959, 1962) has suggested that products of tumour necrosis, and metabolites derived from growing tumour, stimulate reticulo-endothelial cell hyperplasia with the result that the erythroclastic and antibody-forming functions of the spleen and liver, and other organs of the body, are increased. Rigby, Betts, Friedell and Emerson (1962) have come to the same conclusion, namely, that increased reticulo-endothelial cell activity is of major importance in relation to the hæmolytic anæmia of malignancy (and also of infections).

Another way in which tumour mass and necrosis can contribute to anæmia is by hæmorrhage locally into the tumour itself or into its immediate neighbourhood. Experimental work by Price and Greenfield (1958) and Price, Greenfield, Sterling and MacCardle (1959) in rats with various tumours indicated that with some tumours at least hæmorrhage followed by extravascular destruction of erythrocytes was a major cause of apparent hæmolysis. In man, Matthias (1964a) similarly concluded that internal loss of erythrocytes through sequestration in the spleen and in actively growing tumour tissue, as well as loss in the vicinity of the tumour, were important causes of anæmia; and he also stressed the possibility of significant blood loss elsewhere as the result of increased vascular permeability, due to thrombocytopenia, vitamin or protein deficiency.

Reticulo-Endothelial Cell Hyperplasia. As mentioned above, there is evidence for a general stimulation of reticulo-endothelial cell activity in animals with experimentally-induced or transplanted tumours. It seems likely that the stimulation results from the liberation into the blood stream of metabolic products derived from actively growing tumour tissue or from necrotic areas of tumour. In man, reticulo-endothelial cells probably undergo similar hyperplasia in response to actively growing or necrotic tumours (Landaw, 1964). The spleen in particular would then become a more important hæmolytic organ as the result of increased sequestration and phagocytic-cell activity, irrespective of whether the patient's cells were antibody-coated or damaged in any other way.

Friedell (1965), in an interesting review, has suggested that an increased loss of immature erythrocytes by a hyperplastic reticulo-endothelial cell system is an important anæmia-causing factor. Whereas in man in health, a loss of a proportion of immature cells (perhaps because reticulocytes are "sticky") is of no importance, in cancer cases an increased loss may be important, particularly if this is coupled with impaired production. Friedell did not suggest that the avidity of phagocytic cells is increased in cancer, only that an increased number may be present as the result of an increase in work load. The stimulation of phagocytic-cell growth by the products of hæmolysis was also suggested as a way in which a hæmolytic process could become perpetuated.

The role of the spleen in human cases has been studied using the ^{51}Cr technique and evidence for splenic destruction of erythrocytes found. Thus Ultmann (1958) demonstrated splenic sequestration in three out of four patients with disseminated carcinoma. There was no evidence for auto-immunization in these cases and Ultmann considered that the spleen was active in removing from the circulation cells "damaged more subtly" outside the organ. He also demonstrated uptake of ^{51}Cr in a metastasis from a hypernephroma and in hæmorrhagic tumours in the abdomen and liver in a case of myeloma.

Damage to Erythrocytes in or near Tumours. One not uncommon feature of severe hæmolytic anæmia in carcinomatosis is the presence in the circulating blood of distorted erythrocytes and cell fragments. The blood picture then shows the changes of microangiopathic hæmolytic anæmia which Brain, Dacie and Hourihane (1962), in studies mainly on patients in acute renal failure—but including five patients suffering from carcinomatosis—suggested owes its origin to damage to the erythrocytes as the result of being in close contact with the walls of diseased small blood vessels. It seems likely that an analogous mechanism may operate in the carcinoma cases. It is probably significant that whenever hæmolytic anæmia of microangiopathic type has been recorded the tumour has been found at necropsy to be widely disseminated and the bone marrow to be nearly always extensively invaded. Possibly the erythrocytes can be damaged (? the result of a chemical process) if they come in intimate contact with tumour cells, as seems very likely to happen in such a vascular tissue as the bone marrow or when tumour cells invade vascular channels elsewhere in the body. In this connection it is interesting to note that in Stratford and Tanaka's (1965) case many tumour cells were present in the spleen as well as in the bone marrow.

Intra-arteriolar or capillary thrombi have occasionally been found in patients dying of disseminated carcinoma and hæmolytic anæmia (Brain, Dacie and Hourihane, 1962; Stratford and Tanaka, 1965; Brook and Konwaler, 1965). It is possible that the presence of these thrombi may contribute to hæmolysis as is postulated in the acute renal-failure cases and in thrombotic thrombocytopenic purpura (TTP) (see p. 887). Usually, however, the thrombi are few and far between and it is difficult to assess their significance. Certainly, in some cases where a microangiopathic blood picture has been recorded thrombi have been looked for and not found (*e.g.*, by Zittoun and co-workers, 1965). At the other end of the scale is the patient described by Brook and Konwaler (1965), in whom vascular lesions considered to be similar to those of TTP were widespread and found in vessels in the brain, pancreas, lung and gastrointestinal tract—this patient had a carcinoma of the stomach with metastases in lymph nodes, lungs and bones.

Thrombosis in major veins does not seem likely by itself to lead to microangiopathic hæmolytic anæmia. The patient described as Case 24 (p. 782) certainly had many venous thromboses, but he also had disseminated carcinoma. Moreover, widespread venous thromboses, in patients not suffering from cancer, can certainly occur without the complication of hæmolytic anæmia.

The occurrence of hæmorrhage into or around tumours has already been referred to as a potential anæmia-causing mechanism. Some of the extravasated blood cells may return to the blood stream via the lymphatics. Some of these cells may be taken up by erythrophages within lymph-node sinuses; others which pass into the general blood stream may have a reduced survival subsequently. The abnormal and distorted erythrocytes seen in peripheral blood films can be looked upon as cells which have received local damage and are unlikely to survive for their normal life span, either because of metabolic inadequacy or because they are taken out of the blood stream by phagocytic cells in the spleen or elsewhere. Whether adsorption of protein giving weak positive antiglobulin tests can also be attributed to prior damage of erythrocytes by tumour cells, as suggested by Renfer and Hässig (1955), is uncertain.

The hypothesis that erythrocytes in cases of disseminated cancer may sustain significant (? chemical) damage through close contact with cancer cells is not inconsistent with past observations which have demonstrated that the products of autolysis of tumour (or normal) tissue have a lytic effect on erythrocytes (Weil, 1908; Gross, 1948, 1949; Ponder, 1951; Herberman, 1964). In this connection it is worth recording that Friedell (1965) placed first as a causal factor in hæmolysis a direct lytic effect exerted by tumours upon erythrocytes in their immediate vicinity; he mentioned that his own work and that of his colleagues indicated, in hamsters, that a "direct" hæmolytic factor was chiefly present in viable rather than necrotic tumour tissue. He also quoted other work suggesting the presence of increased hydrolytic enzyme activity in the vicinity of tumours and of cytotoxic factors elaborated by tumours which might damage erythrocyte membranes.

Bone-Marrow Invasion. The importance of dyshæmopoiesis as a mechanism leading to hæmolysis is difficult to assess, but in theory at least the presence of many tumour cells in bone marrow could lead to abnormal and defective erythropoiesis by interference with the supply of nutrients essential for normal bone-marrow cell growth. Overt folic-acid deficiency is certainly rare. Dyshæmopoiesis may well, however, be a factor in producing the moderate to marked anisopoikilocytosis seen in the blood films of most patients who are anæmic.

Viruses. Viruses do not seem as yet to have been implicated as a cause of hæmolytic anæmia in human patients suffering from carcinoma or sarcoma. However, as already referred to (p. 788), there is some evidence which suggests that acute hæmolytic anæmia in tumour-bearing rats may be produced in this way.

The Problem of Ovarian Tumours

As described on p. 785, a number of reports of hæmolytic anæmia, usually auto-immune in type, have been reported in patients suffering from ovarian tumours, usually teratomata. It is characteristic that the hæmolytic process ceases and the abnormal antibodies (if present) disappear when the tumour is removed. The nature of the relationship between the tumour and hæmolysis, which appears to be a special one, has not, however, been established.

It is tempting to believe that the teratomatous tumour tissue is reacting against the host and generating antibodies acting on the host's erythrocytes. However, there appears to be no firm evidence in support of this hypothesis. Lympho-reticular tissue, where the antibodies might presumably be formed, has not been reported as being present in conspicuous amounts in the tumour tissue. The alternative hypothesis—that the host is reacting against some component in the teratoma or cyst—lacks supportive evidence, too. Landaw (1964) has, however, recently pointed out that in many patients with carcinoma and hæmolytic anæmia the tumour has been an adenocarcinoma, *i.e.*, a mucin-secreting tumour; he suggested that mucin-like material might be antigenic and lead to the development of antibody which cross-reacted with antigens on the erythrocyte surface. The difficulty in accepting this hypothesis in relation to the carcinoma cases is that auto-antibodies are not as a rule readily demonstrable. It might, however, be applicable to the problem of hæmolytic anæmia and ovarian tumours, where in addition to teratomata, cysts and adenocarcinoma have been implicated, by all of which large amounts of mucin are secreted.

Pathogenesis of Hæmolytic Anæmia in Cancer: A Summary

1. The anæmia of cancer has a complicated origin; excess hæmolysis plays a part in many cases, particularly in the ter-

minal phase of the disease and where the tumour is widely disseminated.

2. There are probably several mechanisms leading to increased hæmolysis: these include hyperplasia of phagocytic reticulo-endothelial cells in the spleen and elsewhere, damage to erythrocytes as the result of circulation within or near tumours, possibly the coating of erythrocytes with abnormal globulins, and the production by the bone marrow of erythrocytes with a shortened life-span as the result of dyshæmopoiesis. Rarely, an auto-immune type of hæmolytic anæmia develops.

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CHAPTER 14

SECONDARY OR SYMPTOMATIC HÆMOLYTIC ANÆMIAS: II

HÆMOLYTIC ANÆMIAS ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS ETC., ULCERATIVE COLITIS, LIVER DISEASE, RENAL DISEASE AND VASCULAR DISEASE

In this Chapter excess hæmolysis developing in the course of certain systemic diseases will be discussed particularly from the point of view of the pathogenesis of the hæmolysis. The underlying disorder itself will not be considered in any detail except in as much as hæmolysis affects the clinical picture.

HÆMOLYTIC ANÆMIA IN SYSTEMIC (DISSEMINATED) LUPUS ERYTHEMATOSUS

Anæmia is a common accompaniment of systemic lupus erythomatosus (SLE). However, although in most cases this is not overtly hæmolytic, auto-immune hæmolytic anæmia of severe grade may occur and even come to dominate the clinical picture. Occasionally, it may be the presenting sign of illness.

Incidence of Overt Hæmolytic Anæmia. Michael, Vural, Bassen and Schaefer (1951) reviewed the hæmatological findings in 111 patients: 102 of them had hæmoglobin concentrations of less than 12 g. per 100 ml. at one time or another; three had overt hæmolytic anæmia. Harvey and his co-workers (1954), in an extensive review, reported on 138 patients, three of whom were known to develop hæmolytic anæmia; they made the point that although almost 80% of the patients were anæmic at one time or another, this was mainly brought about by disturbed erythropoiesis, hæmorrhage, renal insufficiency or infection, rather than hæmolysis. Armas-Cruz and his colleagues (1958), in another review of a large number of patients, stated that 60% were anæmic but that only one had hæmolytic anæmia. Lee and Davis (1959) stated that hæmolytic anæmia was the presenting manifestation in 5% of patients.

None of the patients in Mackay's (1958) and Heinivaara and Eisalo's (1961) series, 35 and 43 in number, respectively, developed overt hæmolytic anæmia although most were anæmic. On the other hand, Videbaek (1962) reported that nine out of 24 successive patients (almost 40%) developed hæmolytic anæmia.

It should be added that the hæmolytic anæmia in almost all the above-mentioned cases was characterized by the presence of a positive direct antiglobulin test. As this is so, those interested in auto-immune hæmolytic anæmia (*e.g.*, Videbaek) are likely to see more examples of hæmolytic anæmia and SLE than general physicians. The present author's laboratory at the Postgraduate Medical School of London has thus studied 15 patients with overt hæmolytic anæmia accompanying SLE: 13 of them were females, 12 being between 10 and 29 years of age and one being aged 40; two were males aged 13 and 52 years, respectively (Table 67). The 15 cases, nevertheless, represent only a small percentage (6%) of about 250 cases of auto-immune hæmolytic anæmia of warm-antibody type studied between 1946 and 1965 inclusive. But the 12 young women represent a large percentage (45%) of all the cases of warm-antibody type (both "idiopathic" and secondary) occurring in females between the ages of 10 and 30 years.

To what extent hæmolysis accounts for the often minor degree of anæmia affecting the majority of patients who develop SLE is not known for certainty. Probably careful erythrocyte survival studies would demonstrate that reduced survival was often a part cause. It is known, too, that positive direct antiglobulin tests may not infrequently be observed with the blood of patients suffering from SLE who do not show signs of overt hæmolysis. The incidence of these positive tests in cases of SLE is, however, not known for certain.

Early Reports of Overt Hæmolytic Anæmia or Positive Antiglobulin Tests

The first reports suggestive of auto-immune hæmolytic anæmia date from 1949. Aegerter and Long referred to two patients whose blood showed marked auto-agglutination. Next, Zoutendyk and Gear (1950) mentioned briefly two patients in whom the direct antiglobulin test was positive. Berman, Axelrod, Goodman and McCloughry (1950) referred to a patient with hæmolytic anæmia but gave no details, and Wiener (1950) mentioned a patient whose serum contained an antibody demonstrable with trypsinized erythrocytes which he considered to be an auto-antibody.

Dubois (1951) referred briefly to three patients who presented with auto-immune hæmolytic anæmia. The direct antiglobulin test was positive. Later, each patient developed the symptoms and signs of SLE, including positive LE-cell tests.

Michael and his co-workers (1951) described three patients with hæmolytic anæmia, in one of whom acute anæmia was the presenting sign. The antiglobulin test was positive in one of two patients in whom the test was carried out. Pisciotta, Giliberti, Greenwalt and Engstrom (1951) reported a further case, also with a positive direct antiglobulin test; this patient's hæmolysis remitted following cortisone treatment. Evans and his co-workers (1951) referred to four patients with SLE all of whom gave positive antiglobulin tests; they were mildly anæmic but none, however, showed signs of active hæmolysis. Marshall, Zoutendyk and Gear (1951), too, described positive antiblogulin tests in three patients with acute SLE.

More Recent Case Reports

Since these early reports many other well-studied cases of auto-immune hæmolytic anæmia developing in patients suffering from SLE have been reported, *e.g.*, by Shearn and Pirofsky (1952), Dubois (1952), Baikie (1953), Harvey and his co-workers (1954), Meacham and Weisberger (1955), Wasserman, Stats, Schwartz and Fudenberg (1955), Gordon and Lowenstein (1955), Grobbelaar (1956), Beikert (1956), Debray and Najean (1958), Dameshek (1958), Boza Sariol and Paisan Adan (1958), Sarles and Levin (1959), Seip (1960), Videbaek (1962), Eisen, Demis and Crosby (1962), Angeloni, Fagiolo and Mastromonaco (1963) and Servi and Restivo (1964).

Clinical and Hæmatological Features

The clinical signs and symptoms and the blood picture in auto-immune hæmolytic anæmia associated with SLE do not seem to differ in any major degree from those in the majority of cases of auto-immune hæmolytic anæmia of the "idiopathic" warm antibody type. (The serological picture is, however, different (see p. 814).) The anæmia may be very severe and hæmoglobinuria may even occur (*e.g.*, Michael *et al.*, 1951; Harvey *et al.*, 1954). Erythrophagocytosis may be seen in peripheral blood films (Meacham and Weisberger, 1955) or in the bone marrow (Angeloni, Fagiolo and Mastromonaco, 1963). Spherocytosis and increased osmotic fragility are commonly found. Erythroblastopenic crises may occur (Sarles and Levin, 1959). The platelet and leucocyte counts may be normal or there may be a leucocytosis in association with acute hæmolytic episodes, but leucopenia and/or thrombocytopenia are not rare.

Seip (1960) recorded the occurrence of SLE in a mother and her infant and postulated that the auto-antibodies had passed across the placenta.

The temporal relationships between the evolution of the characteristic clinical and laboratory signs of SLE and its diagnosis and the occurrence of a hæmolytic anæmia are interesting. Anæmia may precede the onset of any symptoms or signs suggestive of SLE and its diagnosis by many months or even years (Michael *et al.*, 1951; Wasserman *et al.*, 1955; Videbaek, 1962). Videbaek, for instance, cites patients in whom auto-immune hæmolytic anæmia was present for 2, 3 and 6 years, respectively, before SLE was diagnosed. More commonly, although hæmolytic anæmia is the dominant feature from the onset, symptoms suggestive of SLE are also present from the first and the LE-cell test gives positive results (Dubois, 1952; Gordon and Lowenstein, 1955; Meacham and Weisberger, 1955; Cases 25 and 26). In other cases some at least of the signs and symptoms of SLE are established before the onset of clear-cut hæmolytic anæmia.

Association with Thrombocytopenic Purpura. The not infrequent association of thrombocytopenic purpura with auto-immune hæmolytic anæmia has already been referred to ("Evans's syndrome", Evans *et al.*, 1951; Part II, p. 377). The association also occurs in patients who have SLE.

Harvey and his co-workers (1954) mentioned a patient in whom hæmolytic anæmia with hæmoglobinuria appeared long after other features of SLE: splenectomy for "idiopathic" thrombocytopenic purpura had been carried out 2 years before the onset of anæmia. Dameshek (1958) described two cases. The first was a girl aged 10 years: "idiopathic" thrombocytopenic purpura (ITP) developed first, then auto-immune hæmolytic anæmia, and finally joint pains and a positive LE-cell test. The second patient had had splenectomy performed for ITP; the spleen histology suggested SLE, although the LE-cell test was negative. Later, acute auto-immune hæmolytic anæmia developed.

Rabinowitz and Dameshek (1960) referred to three patients, all of whom had had splenectomy for ITP and then developed hæmolytic anæmia. One of them definitely had SLE and this appeared probable in the other two. Rabinowitz and Dameshek made the point that the association of ITP and hæmolytic anæmia should raise a strong suspicion of underlying SLE.

Serological Findings. As already mentioned, it seems that almost all, if not all, cases of severe hæmolytic anæmia developing in patients who have SLE are characterized by a positive direct antiglobulin test. Most writers, including the present author, have therefore regarded the hæmolytic anæmia as being auto-immune in nature.

Typically, the anti-erythrocyte antibodies have been reported as warm ones, and this has been the author's experience. Slightly or moderately raised cold-agglutinin titres were mentioned, however, by Harvey and his co-workers (1954) in three cases and by Videbaek (1962) in one case (titre 256). But the warm antibodies do

Table

Serological Data in 15 Cases of Auto-Immune Hæmolytic Anæmia

Case No. Initials	Sex Age	Antiglobulin Test			Agglutination		
		Direct		Indirect 37° C.	Normal cells (titre)		Papainized normal cells (titre) 37° C.
		Anti-γG	Anti-non-γ		2–4° C.	20° C.	
95 M.R.	F.29	+ +	†	–	16	4	4
98 F.S.	F.17	+ + +	†	+ +	16	4	16
114 B.N.	F.10	+ +	†	+	128	4	4
130 S.P.	F.24	+ +	†	–	16	1	4
137 G.P.	F.23	+ +	†	–	16	< 1	4
148 B.B.	F.26	+ + +	+	+	8	1	64
157 S.M.	M.13	+ +	+	–	1	< 1	1
177 C.E.	F.14	+ + +	+ +	–	1	< 1	4
209 P.D.	F.15	+ + + +	+ + + +	+ +	16	1	4
211 A.B.	M.52	–	±	–	< 4	< 1	1
225 C.C.	F.25	+ + +	+ + +	+ +	8	< 1	8
245 J.C.	F.40	+ +	+ ±	–	...	< 1	4
249 K. de S.	F.19	+ + +	+ + +	+ +	256	4	64
271 P.H.	F.24	+ + +	+ + +	–	16	< 1	8
281 G.S.	F.22	+ + ±	+ + ±	+ + + +	4	1	16

† Specific anti-non-γ serum was not used, but the reaction was only partially inhibited by the addition of human γ globulin to the antiglobulin serum.

* Normal range of serum complement activity = 70–150 units (Dacie and Lewis, 1963).

appear to differ significantly, as judged from their serological reactions *in vitro*, from those found in most cases of auto-immune hæmolytic anæmia of "idiopathic" origin. Thus the antibodies characteristically fix complement and their specificity is still unknown. A specific Rh auto-antibody seems to have been reported only once—by Friedman, Heřmanský and Kučerová (1958)

67

Associated with Systemic Lupus Erythematosus

Lysis				Remarks
PNH cells 37° C.	Papainized PNH cells 37° C.	Papainized normal cells		
		Stored (37° C.)	Fresh (37° C.)	
–	–	–	–	Complement* 21 units
–	+ +	+	+	Complement 15 units; anticoagulant present
–	+	–	–	Complement 7 units
+ + +	...	...	–	...
–	+	+	+	Complement 104 units
+ +	...	–	–	Complement 16 units
–	+ + +	+	±	Complement 57 units
...	...	...	...	Anticoagulant present
+	+ + +	+ + +	+	Complement 45 units
–	+ ±	+ ±	–	...
+ +	+ + +	+ +	±	...
–	±	–	–	...
...	...	+ +	...	...
...	...	+ + ±	...	...
...	...	+ +	...	...

(anti-E); but this antibody was accompanied by a "non-specific" antibody active against enzyme-treated cells.

Fifteen patients have been studied in the author's laboratory. All were suffering from moderate to marked degrees of hæmolysis at the time they were investigated. The direct antiglobulin tests were of the intermediate or "non-γ" type in all these patients and where tests with specific anti-γG and anti-non-γ sera were carried out positive reactions were obtained with both sera (Table 67). Although similar reactions are found in some cases of "idiopathic" origin (see Part II, p. 429), it is interesting to note that a pure anti-γG reaction (the commonest type in "idiopathic" cases) has not yet been encountered in patients with SLE.

The cold-agglutinin titres using normal erythrocytes were normal except in two instances where slightly raised figures (128–256) were recorded; weak agglutination, however, took place at 20° C. in eight instances. Enzyme-treated cells were agglutinated at 37° C. in each case to low or moderate titres; the serum component bringing this about seemed to be a warm antibody and it was probably the same antibody as gave rise to the positive antiglobulin reactions. Enzyme-treated cells, and PNH cells, were generally lysed, as well as agglutinated, at 37° C. Eluates from the patients' erythrocytes were prepared in most instances but in each case the reactions of eluted antibodies, and of the warm antibodies free in serum, lacked apparent specificity.

Serum-complement levels were low or very low in six out of seven cases tested and a circulating anticoagulant was demonstrated in two patients. All in all, the occurrence of an auto-immune hæmolytic anæmia in a young woman, whose erythrocytes give positive antiglobulin reactions with both anti-γG and anti-non-γ sera, whose serum contains antibody agglutinating and hæmolysing enzyme-treated cells at 37° C. and whose serum-complement level is low, is very suggestive of SLE.

Other laboratory findings which point to a diagnosis of SLE, in addition to positive tests for LE cells and antinuclear factor, are positive ("false-positive") serological tests for syphilis, raised serum γ-globulin levels and a markedly raised erythrocyte sedimentation rate (ESR). Videbaek (1962) reported, for instance, a positive Wassermann reaction in four out of nine cases; he also stressed how rapid was the ESR when hæmolysis was active and remarked on the presence, too, in some patients of leucocyte and platelet agglutinins. Anti-leucocyte and anti-platelet antibodies were reported also by Angeloni, Fagiolo and Mastromonaco (1963).

Proneness to develop erythrocyte iso-antibodies may also be a feature of SLE (Callender and Race, 1946, etc., see Part II, p. 593; Kuhns and Bauerlein, 1953), and the occurrence of these iso-antibodies can confuse the issue when attempts are made to identify free auto-antibody in a patient's serum.

The serological abnormalities found in SLE have recently been reviewed by Shulman (1963).

Treatment and Prognosis of Auto-Immune Hæmolytic Anæmia in Association with Systemic Lupus Erythematosus

Hæmolytic anæmias accompanying SLE appear to respond to therapy about as well as do auto-immune hæmolytic anæmias of "idiopathic" type. However, the usefulness of, and justification for, splenectomy have been questioned (see below).

Blood Transfusion. Transfusion should not be withheld in seriously anæmic patients but its value cannot be expected to be more than transient—as in "idiopathic" cases (see Part II, p. 673). The possibly increased likelihood of the patient developing iso-antibodies—which may seriously restrict the practicability of blood transfusion—has already been mentioned.

ACTH and Adrenocorticosteroids. Patients suffering from SLE and auto-immune hæmolytic anæmia seem to respond to steroid therapy at least as well as sufferers from the "idiopathic" disease: this has been clear from the earliest reports in the literature. As mentioned on p. 658 (Part II), it is possible that ACTH by injection may have some advantage over steroids by mouth in certain acutely ill patients and this seemed to be so in two personally studied patients who had SLE (see Fig. 168 (Part II, p. 661), Fig. 206 and Cases 25 and 26). The aim in all patients treated with steroids or ACTH must be to reduce the dose taken to the minimum that will permit a reasonably stable hæmoglobin of, if possible, not less than 10 g. per 100 ml., and that will control at the same time the other manifestations of SLE.

Antimetabolites. The synthesis of 6-mercaptopurine (6-MP) and allied immuno-suppressive drugs has provided new weapons for the control of auto-immune diseases. Whether they have advantages over the adrenocorticosteroid group of drugs is as yet uncertain. They certainly have their drawbacks (see Part II, p. 678), as indeed have the steroids. However, encouraging responses to 6-mercaptopurine and thioguanine have been reported, respectively, by Dameshek and Schwartz (1960)—their patients were, however, not seriously anæmic—and by Eisen, Demis and Crosby (1962).

Splenectomy. Splenectomy has been carried out in patients suffering from SLE for a variety of reasons: for thrombocytopenic purpura; for "hypersplenism", and for hæmolytic anæmia. Some patients have undergone splenectomy (for ITP) before SLE has been diagnosed. Johnson (1953) reported on 12 patients, the basis for the operation being "hypersplenism": nine out of the 12 patients were benefited to various degrees and for various times. None, however, apparently was suffering from severe hæmolytic anæmia.

Dameshek and Reeves (1956), in reporting that three patients with thrombocytopenic purpura or auto-immune hæmolytic anæmia had developed SLE after splenectomy, raised the question as to whether removal of the spleen had precipitated or exacerbated the SLE. This thesis was again propounded by Dameshek (1958) in a paper in which he described two patients in whom SLE was diagnosed within a few months of splenectomy. The postulated harmful role of splenectomy has, however, not gone unchallenged.

Sarles and Levin (1959) recorded, on the contrary, improvement following splenectomy in three patients, while Best and Darling (1962), in an extensive review of 107 cases from the literature and 13 cases of their own, concluded that there was no evidence that splenectomy was harmful in SLE; on the contrary, general improvement in the blood picture, and even general improvement, might be expected. They mentioned that they had found that 34 patients with ITP or auto-immune hæmolytic anæmia had been described as having had splenectomy and as having subsequently developed SLE; but the median time for this was 2½ years after splenectomy and in one-third the SLE had not developed for 5 or more years. Moreover, 35 patients had similarly developed ITP or auto-immune hæmolytic anæmia in association with SLE but had *not* had splenectomy; and the majority of a further 35 patients who developed ITP or hæmolytic anæmia concomitant with or following overt signs of SLE, and then had splenectomy, derived considerable improvement on the whole, both with respect to their cytopenias and, in some patients apparently, even with respect to their other signs of SLE.

Nature of Relationship between Auto-Immune Hæmolytic Anæmia and SLE

Although this type of hæmolytic anæmia is classified (for convenience) as "secondary" or "symptomatic", there is in fact no reason to believe that the hæmolytic process is in any way secondary to the SLE. It is probably more correct to look upon the general phenomena of SLE *and* the hæmolytic anæmia as both having their origin in an increased or aberrant responsiveness of the patient's immune mechanisms.

It may be mentioned that SLE occurs in dogs and that many of the signs and symptoms and consequences closely mimic the human disease. Hæmolytic anæmia is a prominent feature (Lewis, Schwartz and Henry, 1965).

The renal lesions of SLE and positive LE-cell tests have also been found in a small proportion of the NZB/BL strain of mice which develop spontaneous auto-immune hæmolytic anæmia (Helyer and Howie, 1963a, b); and they occur in a high proportion of a cross-bred NZB/BL–NZY/BL strain, in which the incidence of hæmolytic anæmia is relatively low (Helyer and Howie, 1961, 1963a), as well as in NZB/BL–NZW mice (Dubois *et al.*, 1966). These experimental observations are of great interest and underline the close ætiological and pathogenetic relationship between the renal and other phenomena of SLE and the auto-immune hæmolytic anæmia which so often is associated with it.

Prognosis

There is no reason to believe that the prognosis in SLE accompanied by auto-immune hæmolytic anæmia is worse than in SLE without overt hæmolysis. The occurrence of severe thrombocytopenia is, nevertheless, as pointed out by Videbaek (1962), a serious event. According to Best and Darling (1962), more than one-quarter of 35 patients who had SLE and hæmolytic anæmia or thrombocytopenic purpura died of hæmorrhage.

Case Report: Acute Hæmolytic Anæmia Associated with Systemic Lupus Erythematosus

Case 25. A married woman, B. B., aged 26, with two children, was admitted to Hammersmith Hospital on the 28th December 1957 with a history that for the past 18 months she had become progressively unwell and had suffered from periods of weakness and nausea; she had also had intermittent pain and weakness of the fingers and hands, and of her shoulders, knees and hips. For the last 6 months she had become increasingly short of breath and pale and had also suffered from headaches. Her left leg had become swollen as the result of venous thrombosis following the birth of her first child, and this had persisted.

Physical Examination. She was found to be a very pale thin young woman and to be mildly jaundiced and febrile ($T = 100{\cdot}4°$ F.). The liver was not palpably enlarged but the spleen was felt 3 cm. below the left costal margin. A slightly enlarged lymph node was palpable in the left axilla. Her left leg was swollen and there were several fading bruises on the legs but no purpura or petechiæ. A few fundal hæmorrhages were seen. An apical systolic murmur was present and the blood pressure was 115/30 mm. Hg.

Laboratory Investigations. On admission the hæmoglobin was 3·3 g. per 100 ml. with a PCV of 11% and a reticulocyte count of 19%. The total leucocyte count was 4,000 per cu. mm., with 72% neutrophils and 2% metamyelocytes and the platelet count was 197,000 per cu. mm.

Stained films showed considerable anisocytosis and polychromasia, also some microspherocytosis and slight autoagglutination (Fig. 204). A small number of erythroblasts were present. The erythrocyte osmotic fragility was slightly increased: lysis commenced in 0·525% NaCl and the MCF was 0·48% NaCl. The direct antiglobulin test was strongly positive (see below).

The serum bilirubin was 1·6 mg. per 100 ml. The total protein was 7·3 g. per 100 ml., 3·6 g. albumin and 3·7 g. globulins; electrophoresis showed a broad increase in γ globulin. The blood urea was 34 mg. per 100 ml. A small number of LE cells were formed when normal leucocytes were suspended in the patient's serum and the Rose-Waaler (DAT) test was also weakly positive. No evidence was obtained for the presence of a circulating anticoagulant.

The urine was normal.

Serology (Table 67, No. 148). Her blood group was A Rh-negative. The direct antiglobulin test was positive using both anti-γG and anti-non-γ antiglobulin sera, and free auto-antibody was detected in her serum by the indirect antiglobulin method and also by the use of enzyme-treated erythrocytes. The antibody appeared to be "non-specific." PNH erythrocytes were hæmolysed at 37° C. The cold-agglutinin titre was 8 at 2–4° C. and 1 at 20° C. The serum complement activity was subnormal (16 units).

Treatment and Progress. She was treated initially with oral prednisone at a dose of 60 mg. daily, and although this treatment was followed by a rise in reticulocyte and leucocyte counts the hæmoglobin remained at a dangerously low level. On the fourth day ACTH was also given intramuscularly at a dose-rate of 40 mg. b.d.; 2 days later this was increased to 60 mg. b.d. The hæmoglobin commenced to rise on the 6th day and rapidly increased subsequently; the reticulocyte count reached a peak value of 45% by the 7th day (see Fig. 168, Part II, p. 661). The dose of ACTH was gradually reduced; it was withdrawn after 3 weeks and prednisone substituted, at first at a daily dose-rate of 60 mg., which was later reduced to 30 mg. She was discharged from hospital on the 5th February 1958, 5 weeks after admission, with a hæmoglobin of 11·9 g. and 3·4% reticulocytes. The direct antiglobulin test was weakly (1+) positive.

Later Progress. The patient remained well until January 1961 when polyarthritis recurred. This has been controlled with prednisone, but she has had a number of episodes of thrombophlebitis and respiratory infection. These signs and symptoms have persisted (up to January 1966), and she still needs treatment with prednisone. Anæmia, however, has not recurred and the direct antiglobulin test, although weakly positive in July 1959, was negative in January 1961 and has remained negative subsequently.

Summary. A case of systemic lupus erythematosus with severe hæmolytic anæmia as the first serious manifestation. The anæmia remitted on prednisone and ACTH therapy and has not recurred although arthralgia attributable to SLE has persisted.

Case Report: Severe Auto-Immune Hæmolytic Anæmia Associated with Systemic Lupus Erythematosus

Case 26. The patient, P. D., was a young woman aged 15 years. She had been perfectly well until the 22nd February 1962 when she developed an arthralgia which during the course of a week affected her right shoulder, her wrists, the metacarpal-phalangeal joints of her thumbs and her knees. She also complained of frequency of micturition and stated that the urine was dark in colour. On the 27th February 1962 she complained of a sore throat and was found to be febrile and treatment with salicylates and Terramycin was started. On the 1st March pallor and jaundice were noticed. She was admitted to Brighton General Hospital where a diagnosis of acquired hæmolytic anæmia was made. Treatment with oral prednisolone, at first at a dose-rate of 40 mg. daily, then 60 mg., was commenced. She was transferred to Hammersmith Hospital on the 9th March 1962.

Physical Examination. She was found to be pale and slightly

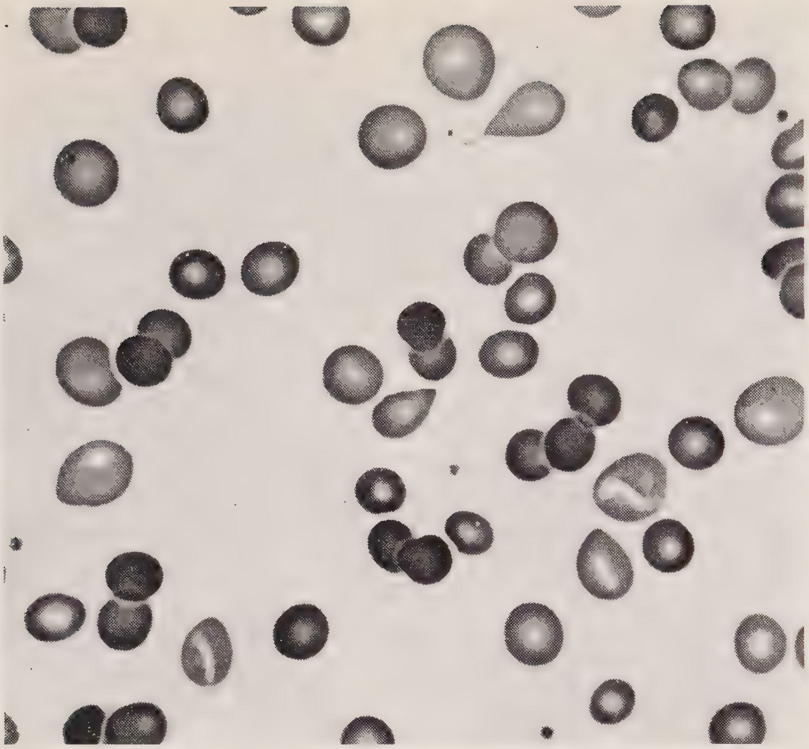

FIG. 204. Photomicrograph of a blood film of a patient suffering from acute auto-immune hæmolytic anæmia associated with systemic lupus erythematosus (Case 25). There is considerable anisocytosis and macrocytosis and slight poikilocytosis, as well as spherocytosis and minimal auto-agglutination. ×700.

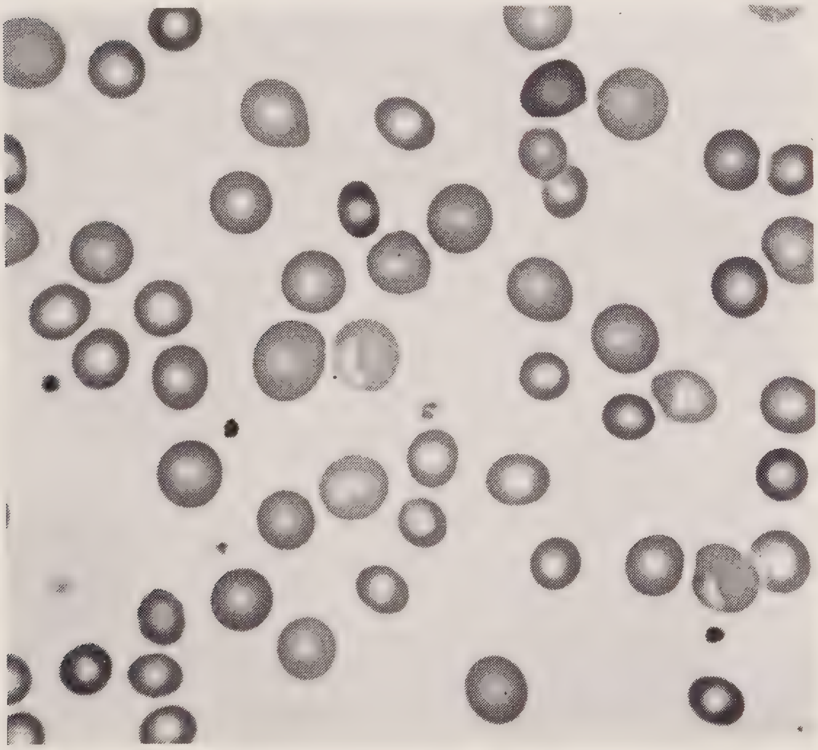

FIG. 205. Photomicrograph of a blood film of a patient suffering from acute auto-immune hæmolytic anæmia associated with systemic lupus erythematosus (Case 26). There is moderate anisocytosis and considerable macrocytosis. ×700.

jaundiced and to be febrile (T = 99–100° F.). The spleen was not palpable and except for a systolic ejection-type murmur physical examination was essentially negative.

Laboratory Findings. On admission, the hæmoglobin was 4·4 g. per 100 ml., erythrocytes 1,100,000 per cu. mm., with 23% reticulocytes. The leucocyte count was 7,000 per cu. mm. with 79% neutrophils and 2% myelocytes; the platelet count was 133,000 per cu. mm. Five erythroblasts per 100 leucocytes were present.

Stained films showed marked anisocytosis, macrocytosis and polychromasia (Fig. 205). LE cells were demonstrated using the patient's whole blood and the test for antinuclear factor was positive. No evidence was obtained for the presence of a circulating anticoagulant. The erythrocyte osmotic fragility was increased: lysis commenced in 0·525% NaCl and the MCF was 0·48% NaCl.

The serum proteins were 7·3 g. per 100 ml., albumin 3·4 g. and globulins 3·9 g. Serum bilirubin was 1·2 mg. per 100 ml. and the blood urea was 36 mg. per 100 ml. The Wassermann and Kahn reactions were positive (and were thought to be "non-specific" in type). The urine was free from protein and no hæmosiderin was detected in the urine deposit. The blood urea was 36 mg. per 100 ml.

Serological Findings (Table 67, No. 209). The direct antiglobulin test was strongly positive using both anti-γ and anti-non-γ sera. The cold agglutinin titre was 16 at 2° C. and 1 at 20° C. Enzyme-treated cells were agglutinated and lysed at 37° C. to low titres. The indirect antiglobulin test was positive. Serum complement activity was slightly subnormal at 45 units (control 150 units). The blood group was A Rh-positive.

Treatment and Progress. The patient's hæmoglobin fell gradually despite raising the dose of prednisolone to 60 mg. daily and on the 14th March, 10 days after commencing treatment, ACTH, at a dose-rate of 60 units intramuscularly daily, was given in addition. The hæmoglobin started to rise on the day following and thereafter rose rapidly, reaching 11·3 g. per 100 ml. by the 30th March (Fig. 206). The dose of ACTH was reduced after 1 week and it was discontinued on the 23rd March. She was discharged from hospital on the 31st March 1962 clinically quite well but still receiving 60 mg. of prednisolone daily. This dose was gradually reduced and prednisolone was finally discontinued on the 9th May 1962.

Her subsequent course has been excellent. Hæmolysis has not recurred; the hæmoglobin has been maintained within the normal range and no signs of overt SLE have developed. When last seen in December 1963 she was still keeping well.

Summary. A case of acute auto-immune hæmolytic anæmia associated with symptoms and signs of SLE. An excellent clinical and hæmatological response followed intramuscular ACTH therapy after treatment with oral prednisolone had apparently failed.

Hæmolytic Anæmia in Association with Polyarteritis (Periarteritis) Nodosa

Dameshek and Rosenthal (1951) referred briefly to four patients whose hæmolytic disease was not benefited by splenectomy and in whom histological evidence of polyarteritis was found *post mortem.* They also described two further possible examples of the syndrome. The first patient gave a positive direct antiglobulin test, but no histological confirmation of polyarteritis was available and this patient may in fact have been suffering from systemic lupus erythematosus. Skin biopsy in the second patient did reveal arteritis but the result of the antiglobulin test was not reported. There is thus little evidence to suggest that the hæmolytic anæmia, when it occurs in association with polyarteritis, is auto-immune in type. It is more probable perhaps that in most cases hæmolysis is "microangiopathic" in origin (see p. 881).

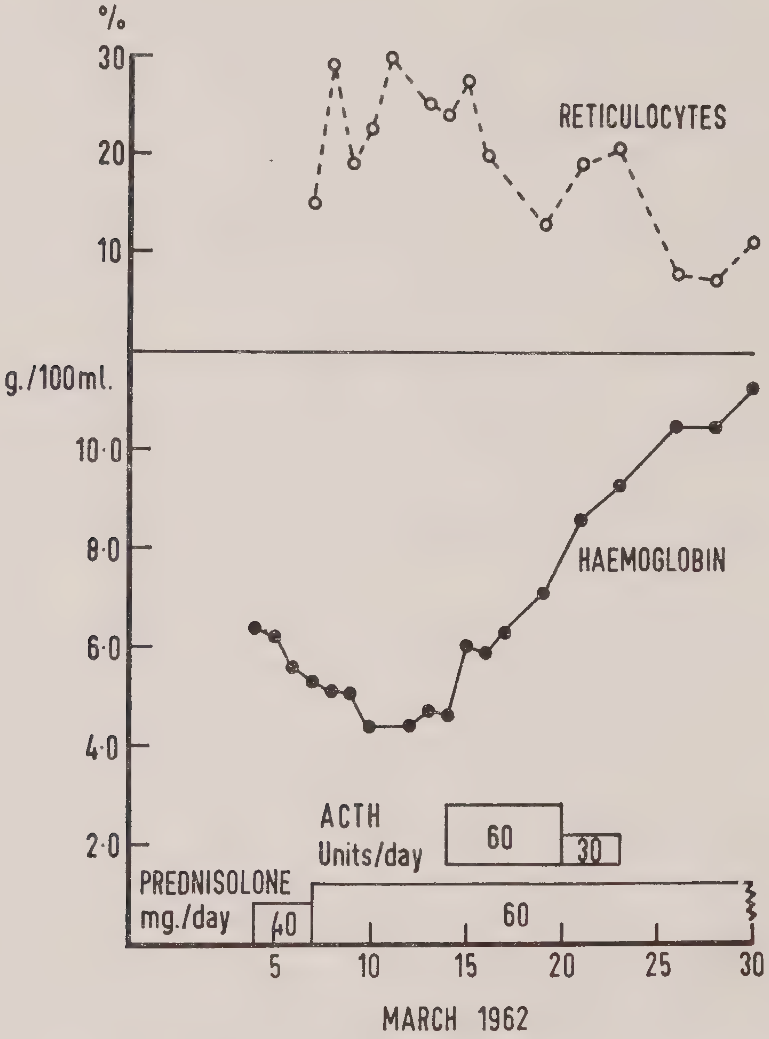

FIG. 206. Response to adrenocorticosteroid and ACTH therapy of a patient suffering from acute auto-immune hæmolytic anæmia associated with systemic lupus erythematosus (Case 26).

Hæmolytic Anæmia in Association with Scleroderma

Fudenberg and Wintrobe (1955) described the occurrence of severe hæmolytic anæmia in a patient who had long-established scleroderma. The direct antiglobulin test was strongly positive and the patient responded to steroid therapy. Whether the association of auto-immune hæmolytic anæmia and scleroderma is more than coincidental is not yet clear.

HÆMOLYTIC ANÆMIA IN ASSOCIATION WITH RHEUMATOID ARTHRITIS

The anæmia so frequently present in active cases of rheumatoid arthritis has been intensively studied, and at one time it appeared as if latent hæmolysis was an important factor in pathogenesis. More recently, however, erythrocyte survival studies have suggested that a significant degree of hæmolysis is an exceptional event. It seems therefore that disturbances in iron metabolism, *i.e.*, dyshæmopoiesis (Ebaugh, Peterson, Rodman and Bunim, 1955; Roy, Alexander and Duthie, 1955; Richmond, Gardner, Roy and Duthie, 1956; Freireich *et al.*, 1957), and in some cases chronic hæmorrhage due to heavy aspirin therapy are the main pathogenetic factors.

Erythrocyte Survival Studies

The original studies were carried out using the Ashby technique. These date back to 1944 when Brown, Hayward, Powell and Witts reported a normal survival of transfused normal blood in one case. Later, Mollison and Patterson (1949) recorded shortened survivals in two patients, 50% of the normal cells transfused being eliminated in about 22 days.

Alexander, Richmond, Roy and Duthie (1956) studied 18 patients and found that the mean "average life span" of transfused normal erythrocytes was 17·6 days, a markedly subnormal figure, and it seemed as if hæmolysis was most rapid in the patients whose disease was most active. Blood from rheumatoid arthritis patients transfused to similar patients survived better. McCrea (1957) in similar studies carried out on 15 patients observed an increased rate of destruction in ten of them, all with very active disease: the curvilinear slope of elimination was held to suggest that extracorpuscular factors were important in hæmolysis. A large and careful study by Freireich and his co-workers (1957) included observations on iron metabolism as well as Ashby survival studies. In 13 patients transfused with normal blood the mean daily erythrocyte loss amounted to 1·46% (range 0·94–2·39%), compared with a normal daily elimination of 0·85–1·0%. The mean daily loss of patient's blood transfused to normal recipients was almost normal, mean 0·97% (range 0·78–1·10%). In the experiments where increased elimination occurred the loss was predominantly exponential, and Freireich and his colleagues concluded that hæmolysis was a random process associated with an abnormal environment rather than due to a defective quality of the erythrocytes. It is interesting to note that in urobilinogen excretion studies the Hæmolytic Index was found to be raised in only five out of 46 tests.

^{51}Cr Studies. The above-mentioned studies, which clearly seemed to point to a relatively frequent occurrence of minor degrees of hæmolysis of transfused normal cells, have not in the main been confirmed by auto-survival studies using the ^{51}Cr technique.

Ebaugh and his colleagues (1955) studied 21 patients and estimated that the mean erythrocyte life-span was 80 days, and Weinstein (1959) found minor degrees of impaired survival in only six out of 13 patients with typical rheumatoid arthritis. Lewis and Porter (1960), who studied 50 patients, found a normal survival in nearly all of them; only four had ^{51}Cr half-times of less than 25 days, the minimum value being 19 days. All four of these patients showed an early loss of about 15% of radioactivity; thereafter, the survival appeared to be normal. Richmond, Alexander, Potter and Duthie's (1961) results were similar: in only six out of 21 patients was the loss of radioactivity marginally greater than normal. Biechl, Stapleton, Woodbury and Read (1962), too, found only slight differences in the mean ^{51}Cr half-times between 20 patients and 20 healthy controls—the minimum half-time was 20 days, in a severely ill patient.

Mongan and Jacox (1964) studied 30 patients whose disease was active; the series included three pairs of identical twins. The patients were transfused with ^{51}Cr-labelled blood from healthy or rheumatoid arthritis donors. In no instance was there a significant shortening of survival which could not be attributed to possible blood-group incompatibility, as for instance in three experiments in which survival was normal for about 2 weeks only to be followed by an abrupt increase in the rate of elimination.

The apparent discrepancy between the data obtained by the Ashby and ^{51}Cr techniques, respectively, requires an explanation. In fact, similar observations have been made in cases of auto-immune hæmolytic anæmia (Part II, p. 387). In ^{51}Cr auto-survival studies, where there is an extrinsic destructive process, a population of cells is labelled which has already been exposed to an abnormal environment and which contains an increased proportion of resistant cells. Many of the most sensitive cells have probably already been eliminated, and one labels, too, a population which contains a raised proportion of young cells. Whether this is the complete explanation for the discrepancies remains to be seen.

It should be emphasized that in rheumatoid arthritis an increased rate of erythrocyte destruction, even if present, is seldom if ever severe enough to be reflected in clear-cut signs of hæmolysis such as jaundice, increased pigment excretion and marked reticulocytosis. The foregoing remarks, and indeed the whole discussion, refers to uncomplicated rheumatoid arthritis. Cases of arthritis with splenomegaly (Felty's syndrome) or arthritis with systemic lupus erythematosus are excluded.

Auto-immune hæmolytic anæmia occurring as a possible complication of rheumatoid arthritis is rarely found. Only two among the author's series of about 250 patients suffering from various types of auto-immune hæmolytic anæmia gave a history of rheumatoid arthritis, an association which could be coincidental; and it seems likely that if a patient gives a history of joint symptoms and is found to have hæmolytic anæmia, the ultimate diagnosis in most instances will be systemic lupus erythematosus.

Hæmolytic Anæmia in Association with Acute Rheumatic Fever

Reinhold (1954) transfused eight children suffering from acute rheumatic fever with normal blood and studied its survival by the Ashby method. The mean erythrocyte survival appeared to be 20 days and the slope of elimination was curvilinear.

Major degrees of hæmolysis are clearly most exceptional but one example may be quoted. Giraud and his co-workers (1955) reported the occurrence in a 9-year-old boy of severe auto-immune hæmolytic anæmia 1 year after an attack of acute febrile polyarthritis associated with chorea.

More recently, Hersko and Izak (1965) have published the results of a large survey carried out in Israel. Thirty-six patients, mostly children, were studied in detail: their hæmoglobins ranged from 6·2 to 15·0 g. per 100 ml. and their reticulocytes counts from 0·6 to 4·4%. The mean ^{51}Cr T$\frac{1}{2}$ was 18·7 days (range 5–27·5 days) when the patients' own erythrocytes were labelled. Transfused normal cells survived normally in four experiments and the authors concluded that increased hæmolysis, if present, was the result of an intracorpuscular defect. The antiglobulin test was negative. Steroid therapy had a beneficial effect on the patients' anæmia.

AUTO-IMMUNE HÆMOLYTIC ANÆMIA ASSOCIATED WITH ULCERATIVE COLITIS

In recent years several patients have been described in whom ulcerative colitis has apparently been complicated by hæmolytic anæmia of the warm auto-antibody type.

Lorber, Schwartz and Wasserman (1955) referred to four patients: the first showed little evidence of hæmolysis, yet the direct antiglobulin test was strongly positive and the serum contained a pan-antibody and an auto-anti-e as well as iso-anti-D and iso-anti-E. The patient's genotype was *cde/cde*. The second patient, too, showed little evidence of hæmolysis, but her erythrocytes also gave a positive antiglobulin test; the serum contained a panagglutinin against enzyme-treated cells. The third patient developed overt hæmolytic anæmia at a time when the colitis was quiescent. The direct antiglobulin test was strongly positive and there was marked spherocytosis; the serum contained iso-anti-E and a pan-antibody. Splenectomy was followed by incomplete remission. The fourth patient's erythrocytes gave a weakly positive antiglobulin test but the serum contained a pan-antibody as well as iso-anti-E and iso-anti-Kell. Lorber and his colleagues suggested that sensitization might somehow be brought about by the presence of the diseased bowel. They noted in two, and possibly in three, of their cases that the antiglobulin reactions became weaker after colectomy.

Balint, Hammack and Patton (1962) in a brief report described a further patient with hæmolytic anæmia and in whom specific auto-antibodies were demonstrated (anti-e + f). No cross-reactivity between colonic antigens and the erythrocyte antibody could be demonstrated. Colectomy was carried out and the antiglobulin test became negative in 6 weeks. It was suggested that the auto-antibodies could have developed as the consequence of the diseased colon allowing the absorption of altered erythrocyte antigens.

Fong, Fudenberg and Perlmann's (1963) patient gave a strongly positive direct antiglobulin test, but the auto-antibody lacked apparent specificity. Erythrocytes failed to cross-react with the antibodies to colonic extract, which were present in high titre, and colonic extract used as antigen failed to react with erythrocyte antibody. Fong and his co-workers suggested that the most likely explanation for the occurrence of auto-immune hæmolytic anæmia in association with ulcerative colitis is a genetically determined predisposition to form auto-antibodies. If this applies to iso-antibody formation too, then the occurrence of iso-antibodies in three out of the four patients of Lorber, Schwartz and Wasserman is in accord with this concept.

Further cases have been described by Gastaldi, Regazzini, Chiappino and Fedeli (1963), whose patients gave positive tests for anti-colon antibodies as well as for anti-erythrocyte antibodies, and by Molaro and Barillari (1964), whose patient gave a strongly positive antiglobulin test although there appeared to be no evidence for excess hæmolysis at the time.

The present author has encountered two examples of auto-immune hæmolytic anæmia associated with ulcerative colitis out of a total of about 250 cases of auto-immune hæmolytic anæmia investigated, as well as a further patient whose erythrocytes gave a clear-cut positive direct antiglobulin reaction although there was no convincing evidence of excessive hæmolysis.

The above-mentioned case histories are, in the author's view, of great interest and importance: they are exceptions which when more fully understood may be expected to help to "prove the rule" with respect to the ætiology and pathogenesis of cases of auto-immune hæmolytic anæmia which at present have to be classified as "idiopathic". As with the alpha-methyldopa cases (see Chapter 16, Part IV), the demonstration that the auto-antibodies often have Rh specificity appears to be of particular significance.

HÆMOLYTIC ANÆMIA ASSOCIATED WITH HEPATITIS OR CIRRHOSIS OF THE LIVER

Anæmia in liver disease has been the subject of much speculation and research, for although it is not often severe in degree—except perhaps as the result of hæmorrhage—it is frequently present.

Like the majority of secondary anæmias several factors play a part in its causation and, leaving aside blood loss by hæmorrhage, defective bone-marrow function and increased hæmolysis may both be important. There is an extensive literature (see Marks, De Bellis and Burka, 1963; Pitcher and Williams, 1963) and there has been much discussion—particularly in the German literature—on the part hæmolysis may play in post-hepatitic hyperbilirubinæmia.

The following review is concerned solely with the occurrence and significance of increased hæmolysis. Historically, the early papers on liver disease and anæmia, and the possible role of hæmolysis, were inextricably tied up with the problem of "Banti's disease", but the idea that increased blood destruction was a causal factor in the anæmia accompanying many types of liver disease, and that this took place mainly in the spleen, dates back many years (see Eppinger, 1913). It was not until the 1930's, however, that case reports were published of patients in whom the laboratory data suggested that overt hæmolysis was occurring (Fellinger and Klima, 1933–4; Sjovall and Wallgren, 1934; Hijmans van den Bergh and Kamerling, 1935; Rosenberg and Walters, 1936; Watson, 1937, 1939). It now seems clear that increased hæmolysis may be present to varying degrees in at least most types of liver disease. For convenience, its occurrence in acute and subacute hepatitis will be considered separately from that in the various forms of cirrhosis.

Acute and Subacute Hepatitis

Overt hæmolysis is not common in viral hepatitis, but there is by now quite a large literature on its occurrence. Generally, in the cases where there is significant excess hæmolysis, signs of this begin to appear only after the hepatitis has been established for some weeks. Very occasionally the direct antiglobulin test is positive (see p. 835).

Sjovall and Wallgren (1934) described the presence of severe anæmia complicating hepatitis in a boy suffering from Wilson's disease; they mentioned, too, that they had observed a similar sequence in some other cases of hepatitis in children. A further example of severe hæmolytic anæmia in a child was reported by Kjellberg (1941); this became obvious about 1 month after hepatitis had been diagnosed. Neumann and Hommer (1949) mentioned three patients in whom signs suggestive of hæmolysis developed also about 1 month after the commencement of the hepatitis. Three further examples were reported by Hirscher (1950) in cases of acute or subacute hepatitis.

Bakx, van Loghem and Klomp-Magnée (1953) described the development of jaundice and anæmia in an infant which they thought was secondary to infective hepatitis; the direct antiglobulin test was weakly positive and cold agglutinins were present in the serum.

Bornemann and Michel (1955) reported on studies carried out on 40 patients who had suffered from epidemic hepatitis. No overt examples of hæmolysis were discovered but hæmatological signs which they interpreted as suggestive of minor degrees of hæmolysis were found in 12 patients. The direct antiglobulin test was positive in one instance.

Kalk and Wildhirt (1955), too, observed minor signs of hæmolysis in about one-third of 150 patients who presented with signs of post-hepatitic hyperbilirubinæmia. Horváth and Iványi's (1959) data were very similar: twenty-four of 98 patients who suffered from acute viral hepatitis developed reticulocyte counts exceeding 3·0% and 23 were thought to show minor degrees of increased osmotic fragility.

A further more recent statistical survey has been published by Fodor and Tănăsescu (1962), who found that 2% out of a total of 1,200 patients had developed signs of hæmolysis following hepatitis, and that the incidence of hæmolysis in patients suffering from chronic hepatitis was as high as 10%. Twenty-eight patients gave a positive direct antiglobulin test.

The results of some ^{51}Cr erythrocyte survival studies have recently been published by Frick and Schmid (1965). Seventeen patients with acute hepatitis and five suffering from acute exacerbations of chronic hepatitis were studied: the ^{51}Cr T$\frac{1}{2}$ was subnormal (15–23 days) in ten patients of the acute hepatitis group and in four of the patients who were suffering from chronic hepatitis (^{51}Cr T$\frac{1}{2}$, 10–20·5 days). None of the acute hepatitis patients was, however, anæmic and the episodes of hæmolysis seemed to be of brief duration and well compensated.

Details of further individual patients suffering from hæmolytic anæmia accompanying hepatitis have been described by Dacie (1954, p. 362), Hennemann (1954), Freire and Menano (1955), Wasserman, Stats, Schwartz and Fudenberg (1955), Beickert and Siering (1958), Raffensperger (1958), Kölle (1960), Kivel (1961), Zamaraeva and Tishina (1963), Sansone, Rovei and Rasore-Quartino (1963) and Hansberger and Bong Hak Hyun (1963). Most of these case reports contain points of unusual interest.

In the patient of Wasserman and his colleagues (1955) the signs of increased hæmolysis were noticed on the 27th day of illness. A hæmolytic factor active at 37° C. was demonstrated in the patient's serum at this time; it disappeared on the patient's recovery.

Beickert and Siering's (1958) patient developed an immunopancytopenia 6 weeks after the onset of hepatitis; leucopenia was severe; the platelets were absent and the patient suffered from a hæmorrhagic state. Direct and indirect antiglobulin tests carried out on erythrocytes were both positive.

Kölle's (1960) patient developed severe hæmolytic anæmia following typical hepatitis. The antiglobulin test was at first negative, but it became positive later and the cold-agglutinin titre rose to 2,000. Incomplete warm auto-antibodies were thought to have been developed also. Kivel (1961), in a review of blood changes in acute viral hepatitis, described two patients, one who developed a pancytopenia and the other who showed signs of marked hæmolysis. The incident in the latter patient may, however, have been an exacerbation of an underlying mild hereditary non-spherocytic hæmolytic anæmia.

The patient of Sansone, Rovei and Rasore-Quartino (1963) may also have been at increased risk because of a hereditary enzyme defect—G-6-PD deficiency: he developed an acute hæmolytic anæmia and severe jaundice; the serum bilirubin rose to 33 mg. per 100 ml. (15·6 mg. conjugated) and the reticulocyte count to 50%.

Post-Hepatitis Hyperbilirubinæmia. Despite the considerable number of case reports now available of overt hæmolysis following hepatitis (see above), it is clearly not a common event. A raised level of unconjugated bilirubin in the plasma, which persists for a variable length of time, is, however, not infrequent after hepatitis and there is a large literature (predominantly German) which is concerned with the part, if any, played by excessive hæmolysis in its causation.

All writers seem to agree that hæmolysis is not in fact a factor in the causation of the common type of intermittent post-hepatitic hyperbilirubinæmia—but they do not deny that occasionally a genuine hæmolytic anæmia may develop (Schmidt, 1952; Goldeck and Küchmeister, 1955; Kalk and Wildhirt, 1955; Hennemann, 1956–7; Frenger, Scheiffarth and Ezzedine, 1959; Libánský, 1960). The hyperbilirubinæmia appears likely in most instances to be the result of an abnormality of liver-cell function or occasionally to continuing hepatitis.

Cirrhosis

There is a large literature on increased hæmolysis in cirrhosis. Excess hæmolysis is in fact frequent, but it is usually not severe. The early reports of Hijmans van den Bergh and Kamerling (1935), Rosenberg and Walters (1936) and Watson (1937, 1939) have already been mentioned (p. 827).

Rosenberg and Walters found raised reticulocyte counts in the majority of patients suffering from various types of liver disease: almost all the patients with counts exceeding 2% had cirrhosis. Watson (1937) referred to seven patients with cirrhosis in whom there was clinical evidence of hæmolysis; the fæcal urobilinogen excretions varied from 319 to 1,257 mg. per day. In his 1939 paper Watson mentioned another similar patient, making a total of eight clear examples of increased hæmolysis encountered in a group of 59 patients with liver disease; 38 of them were considered to have cirrhosis and 21 "catarrhal jaundice".

Other probable or clear-cut examples of hæmolytic anæmia in patients in whom cirrhosis has been diagnosed in life or *post mortem* were described by Davidson and Fullerton (1938), Voit (1948), Coleman (1948), Hahn and Lüttgens (1949), Hyman and Southworth (1951), Cattan and co-workers (1952), Arrau and Matte (1953), Ruggieri and Bolognesi (1955), Jandl (1955), Hennemann (1956, 1956–7), Dureux and co-workers (1963) and Smith, Lonergan and Sterling (1964).

Hyman and Southworth (1951) mentioned eight patients with liver disease who had overt hæmolytic anæmia; four had cirrhosis and four focal necrosis of the liver. Three of the patients with cirrhosis gave positive direct antiglobulin tests. Arrau and Matta (1953) recorded that two out of 18 cirrhotic patients gave positive direct antiglobulin tests and had agglutinins in their sera (? against enzyme-treated erythrocytes) which reacted at 37° C. Cold agglutinins were said to be present in other patients at increased titres. Ruggieri and Bolognesi (1955) described four patients with cirrhosis and probable hæmolytic anæmia and reported that the direct antiglobulin test was positive in two and that high-titre cold agglutinins were present in the serum of a third patient.

Jandl (1955) studied 20 patients with alcoholic cirrhosis extensively. All except one were anæmic and evidence for increased hæmolysis was obtained by Ashby survival studies in all of them (see below). None of the patients gave positive direct antiglobulin reactions. Four patients were found to be folic-acid deficient and others showed signs of a diminished capacity for hæmoglobin synthesis.

Smith, Lonergan and Sterling (1964) have more recently reported under the title "spur-cell anæmia" studies on a patient suffering from alcoholic cirrhosis. This patient had a moderately severe hæmolytic anæmia and thrombocytopenia (hæmoglobin 6·7 g. per 100 ml., with 6% reticulocytes, and 40,000 platelets per cu. mm.), and 80% of the erythrocytes had spur-like structures (2–4μ in length) projecting from their surface. There were no signs to suggest that the patient was suffering from a complicated congenital abnormality, *i.e.*, acanthocytosis. Normal erythrocytes transfused to the patient were found to develop spurs *in vivo* after 1 week and also after 3–18 hours' incubation in the patient's plasma *in vitro*. It was concluded that the patient, as the result of his liver disease, was producing an abnormal plasma constituent which seemed likely to be a protein or some substance bound to protein.

Erythrocyte Survival Studies. A great mass of data are now available as the result of Ashby and radioactive chromium studies, and all authors agree that an increased rate of cell destruction can often be demonstrated in patients suffering from liver diseases, particularly in cirrhosis which has been most widely studied. The increased rate of elimination applies in most cases to normal erythrocytes as well as to those of the patient. Increased splenic uptake of radioactivity has sometimes been demonstrated and there is some evidence relating the size of the spleen to the extent of the hæmolysis.

The first reports date from 1951, when Sheets, Janney, Hamilton and DeGowin (1951) reported a mean cell life-span for normal erythrocytes of 28 days before splenectomy, and 115 days after splenectomy, in a patient suffering from post-necrotic cirrhosis. Then Schlegel and Böttner (1952) gave 81 days as the mean survival (only slightly subnormal) in 19 patients, with unspecified liver diseases, who were transfused with normal blood. Next, Chaplin and Mollison (1953) reported mean survivals of normal erythrocytes of 25–55 days in five patients who had cirrhosis.

Jandl's (1955) work has already been mentioned: the half-life of normal transfused cells was 5–49 days in 16 patients, compared with the normal of 52–68 days (Ashby method). Jandl mentioned, too, that studies with ^{51}Cr indicated that the spleen played a major role in erythrocyte destruction (but see Pitcher and Williams (1963), p. 832).

Subsequent workers have used ^{51}Cr almost exclusively. Capps, Weinstein, Ettinger and Jones (1954) and Jones, Weinstein, Ettinger and Capps (1955) reported on 19 patients, 15 being cirrhotics: eight of the 19 patients showed a decreased ^{51}Cr T$\frac{1}{2}$ when their own cells were labelled and four gave results at the lower limits of normal. Allen, Carr and Klotz (1957) in similar studies found evidence for a slight decrease in erythrocyte survival time in six out of 12 patients, all with cirrhosis. Cawein, Hagedorn and Owen (1958, 1960) studied 32 patients and controlled their results by testing the radioactivity of the stools for signs of occult bleeding: twenty-eight of the patients gave ^{51}Cr half-times of less than 25 days (their lower limit of normal) and only four bled significantly from the gastrointestinal tract.

Other studies illustrating the frequency of minor to moderate decrease in erythrocyte survival time in cases of cirrhosis have been published by Pelnard-Considère, Sarrazin, Canivet and Fallot (1958), Fauvert, Loverdo, Nicollo and Boivin (1958), Hall (1960), Sheehy and Berman (1960), Combrisson (1960), Boivin, Hartmann and Fauvert (1961), Pitcher and Williams (1963), Boivin, Nicollo and Fauvert (1964), Katz, Velasco, Guzman and Alessandri (1964), Prato and colleagues (1965), Frick and Schmid (1965) and Kimber, Deller, Ibbotson and Lander (1965).

Fauvert and his colleagues (1958) studied six patients with viral hepatitis in addition to 40 with cirrhosis: the ^{51}Cr T$\frac{1}{2}$ was subnormal in 32 of the cirrhotic patients (8–21 days); it was also lowered (12–21 days) in the patients with hepatitis. Intracorpuscular as well as extracorpuscular factors were thought to be implicated. Hall (1960) and Combrisson (1960) came to a similar conclusion. Combrisson's study included nine patients suffering from hæmochromatosis: eight of them had a reduced erythrocyte survival (^{51}Cr T$\frac{1}{2}$ 5–24 days, mean 17 days).

Sheehy and Berman's (1960) data included figures for plasma volume —which they found to be increased in 15 out of 24 cirrhotic patients. Similar figures for plasma volume were given by Boivin, Hartmann and Fauvert (1961): their results, based on a large series of patients, also illustrate that diminished cell survival may be found in all types of cirrhosis.

Pitcher and Williams (1963) also investigated patients suffering from various type of liver disease as well as patients with cirrhosis; in particular, shortened cell survivals were found in infective hepatitis (17 out of 18 patients), in all of 10 patients with obstructive jaundice and even in two of five patients thought to be suffering from Gilbert's congenital hyperbilirubinæmia. Surface counting was carried out over the spleen in 10 patients and evidence for accumulation of radioactive chromium was detected in two of them. An extracorpuscular mechanism was thought to be the major cause of the hæmolysis.

Boivin, Nicollo and Fauvert (1964) used $DF^{32}P$. Eleven cirrhotics were investigated and the mean cell life-span was found to be 52–87 days in 10 of them; in six it was less than 70 days. The eleventh patient had an overt hæmolytic anæmia: his elimination curve was complex, being partly linear and partly exponential.

Katz and co-workers (1964) studied 34 patients in all, with acute or chronic hepatitis (15), cirrhosis (13) or extrahepatic obstructive jaundice (6). All but three had a reduced $^{51}Cr\ T\frac{1}{2}$ (minimum 12·5 days). Steroid therapy was found not to influence hæmolysis and there appeared to be no correlation between the $^{51}Cr\ T\frac{1}{2}$ and the serum bilirubin or hæmoglobin levels or with tests of liver function. They concluded that extracorpuscular as well as intracorpuscular hæmolytic factors were at work.

Prato and co-workers (1965) carried out some cross-transfusion experiments using ^{51}Cr in patients with cirrhosis: their results, too, suggested that intracorpuscular and extracorpuscular factors both played a part in the diminished erythrocyte survival.

Kimber, Deller, Ibbotson and Lander (1965) have recently published the results of a study carried out on 24 patients, the majority suffering from alcoholic cirrhosis. Erythrocyte survival was reduced in 18 of them: the $^{51}Cr\ T\frac{1}{2}$ was 9 days in one patient but in the others it was less markedly shortened (15–23 days). Surface counting was carried out in 21 patients, but in only three did there appear to be excess accumulation of radioactivity in the spleen. However, the mean $^{51}Cr\ T\frac{1}{2}$ was lower in the patients whose spleens were palpable compared with those whose spleens were not palpable (17·3 days compared to 22·6 days).

"Zieve's Syndrome"

Zieve in 1958 described what he considered to be a hitherto unrecognized syndrome—jaundice, hyperlipæmia, hypercholesterolæmia and hæmolytic anæmia developing as a transient consequence of heavy drinking in chronic alcoholics. The excessive hæmolysis was slight to moderate and of brief duration and the minimum hæmoglobin level in 20 patients was reported to be 8·3 g. per 100 ml. The average decrease in hæmoglobin was 2·9 g., and the average maximum reticulocyte count 9%. Maximum changes were observed in the 1st–2nd week of the episode. Spherocytes were noted in the blood films in eight patients and increased osmotic fragility was recorded in seven. The greater part of the raised level of serum bilirubin was due to retention of unconjugated pigment.

Liver biopsy in these patients (five cases were described in detail) showed fatty infiltration with or without moderate portal cirrhosis, and the hæmolysis was attributed to the hyperlipæmia and possibly to the liberation of lipase into the blood and the transformation of lecithin to actively lytic lysolecithin. The basis of the disease was thought to be a transient disturbance of hepatic function.

In later papers, Zieve and Hill (1959, 1961) contrasted the findings in "hyperlipæmic hæmolytic anæmia" with those in "cirrhosis with hypersplenism." In the former the hæmolytic anæmia is transient, the illness brief, the liver is large, and cirrhosis is not prominent; fatty infiltration is the essential ingredient. Jaundice is moderate or severe, serum cholesterol is high and there may be frank lipæmia; osmotic fragility is usually increased. In the latter, mild to moderate anæmia is persistent, splenomegaly is prominent, the serum bilirubin is only moderately raised and osmotic fragility is not increased.

Further patients presenting the features of "Zieve's syndrome" have subsequently been reported by Haber (1961), Myerson and Gilbert (1962), who, too, attributed the onset of their patients' illness to an acute hepatocellular dysfunction, Blass and Dean (1966), and others. Blass and Dean concluded, however, that liver failure, hyperlipæmia, hæmolytic anæmia and pancreatitis all occurred independently in alcoholic cirrhosis: *in vitro*, they were unable to demonstrate that their patients' plasma had any unusual lytic properties despite a raised content of triglycerides, phospholipids and lysolecithin.

Wilson's Disease (Hepatolenticular Degeneration)

A small number of cases have been described in which episodes of hæmolysis have developed in patients suffering from Wilson's disease. As already mentioned (p. 827), Sjovall and Wallgren (1934) had described the occurrence of severe anæmia in a boy suffering from Wilson's disease. More recently, in a review of 20 cases, Walshe (1962) mentioned briefly that in three of them the jaundice appeared to be both hepatic and hæmolytic in origin; in one patient Lederer's anæmia had been diagnosed.

Turpin and co-workers (1963) referred to a boy aged 10 years who developed an acute hæmolytic episode of obscure origin; he recovered from this within a few weeks but became jaundiced later. His liver and spleen were then found to be enlarged and there were signs of disturbed liver function. Two years later signs of Wilson's disease appeared at a time when the anæmia had disappeared. A rather similar occurrence was more recently described by Carr-Saunders (1965). A girl aged 10 years developed severe anæmia and a high reticulocytosis; the direct antiglobulin test was negative; her liver was palpable. She was later reinvestigated and found to be suffering from Wilson's disease. (An elder sister had died, when aged 7 years, of an illness accompanied by jaundice and anæmia; nodular cirrhosis was found at necropsy.)

The present author has investigated one patient who suffered from two transient but acute episodes of hæmolytic anæmia and who later developed the classical symptoms and signs of Wilson's disease. This patient is a young man who as a boy aged 9 years developed acute hæmolytic anæmia, apparently associated with a Salmonella infection;

five months later a further acute episode appeared to follow vaccination against smallpox. He made a good recovery from these attacks and hæmolytic anæmia has not recurred. Two years later, however, neurological signs appeared for the first time and progressed relentlessly; eventually Wilson's disease was diagnosed (Dr. J. M. Walshe, personal communication).

The relationship between hæmolytic anæmia and Wilson's disease seems at the moment to be a complete mystery; and the occurrence of acute hæmolytic episodes well before the appearance of signs of overt Wilson's disease or cirrhosis is particularly remarkable.

Hæmatological Findings in Hæmolytic Anæmia in Liver Disease

The blood picture in cases of liver disease where there is evidence of increased hæmolysis is not a very distinctive one. There is usually some degree of macrocytosis and mild anisocytosis, hypochromia and poikilocytosis. Spherocytes may be seen in some cases (*e.g.*, in "Zieve's syndrome" and where there is an autoimmune basis) but it is seldom a striking feature. Irregularly-contracted cells have been seen in patients who have severe hepatic necrosis (see Case 24 of Dacie (1954, p. 362)), and acanthocytes, cell fragments and arc-shaped and triangular cells were reported by Dreyfus (1965) as being present in the blood of a patient suffering from severe alcoholic cirrhosis who had a moderate anæmia, reticulocytosis and thrombocytopenia.

Target cells may be seen in some cases, particularly in hepatitis and biliary obstruction. Polychromasia will be seen according to the reticulocyte count—which will vary with the intensity of hæmolysis; punctate basophilia is not a conspicuous feature. Rouleaux formation is often excessive; true autoagglutination is rarely seen.

Osmotic fragility is increased in the presence of spherocytosis. More often than not it is normal (*e.g.*, Jandl, 1955), but striking increases have been noted in a few cases (*e.g.*, Kjellberg, 1941; Hirscher, 1950), and in "Zieve's syndrome" (Zieve, 1958; Zieve and Hill, 1959).

The leucocyte count depends to some extent upon the nature of the underlying liver disease. Splenomegaly is often accompanied by leucopenia, but neutrophil leucocytosis may develop in acute liver necrosis (*e.g.*, Case 24 of Dacie (1954, p. 362)). The platelet count is usually normal or reduced.

Serological Findings. The direct antiglobulin test is negative in the vast majority of patients suffering from liver diseases, even in the presence of overt hæmolysis. Nevertheless, a small number of positive tests have been recorded, for example, by Hyman and Southworth (1951), Hennemann, Möller and Gillert (1952), Bornemann and Michel (1955), Ruggieri and Bolognesi (1955), Hennemann (1956–7), Beickert and Siering (1958), Kölle (1960) and Easton, Priest and Giles (1965). In most instances, unfortunately, the serological reactions have not been given in detail.

The patients reported by Hennemann, Möller and Gillert, Bornemann and Michel, Ruggieri and Bolognesi, Beickert and Siering, and Kölle as giving positive antiglobulin tests all had epidemic hepatitis. High titres of cold agglutinins were recorded in one of Ruggieri and Bolognesi's cases and in Kölle's patient. The serum of Easton, Priest and Giles's patient, who had cirrhosis, contained a strong auto-antibody of wide thermal amplitude which agglutinated and hæmolysed enzyme-treated cells at 37° C. if they had been stored. The direct antiglobulin test was strongly positive with an anti-non-γ-globulin serum and weakly positive with an anti-γG-globulin serum.

Pathogenesis of Increased Hæmolysis in Liver Disease

There are probably several mechanisms which contribute to increased hæmolysis in liver disease: *e.g.*, damage to the erythrocytes during passage through the diseased liver; splenomegaly leading to increased splenic sequestration; and, rarely (apparently), the formation of anti-erythrocyte auto-antibodies. All these mechanisms are "extracorpuscular" and would lead to hæmolysis of transfused normal cells—as has commonly been observed (see p. 831).

Damage to Erythrocytes in the Liver. The idea that erythrocytes could be damaged by circulating through diseased liver tissue is speculative, but it seems at least reasonable to the author to suppose that in acute hepatitis or liver necrosis products of parenchyma-cell autolysis might affect passing erythrocytes deleteriously. This is in line with the hypothesis put forward as an explanation for microangiopathic hæmolytic anæmia (see p. 887). It seems likely, too, that in "Zieve's syndrome" the erythrocytes may be damaged by the liberation of lysophosphatides and fatty acids originating in the liver (Jeannet and Hässig, 1964) and that the damage to the erythrocytes which leads to their eventual lysis takes place predominantly during their passage through the liver. In this connection it is interesting to recall that Moser (1949) observed an increased percentage of microcytes in liver-puncture blood in cases of hepatitis and suggested that hæmolysis was being brought about by fragmentation. Kivel (1961), too, speculated that hæmolysis might be brought about by lytic enzymes liberated from autolysing liver cells. Experimentally, there is some evidence that the intravenous injection of various fatty emulsions is probably associated with increased hæmolysis (Loewy, Freeman, Marchello and Johnson, 1942; Creditor, 1953; Levenson, Upjohn and Sheehy, 1957).

Splenomegaly. Increased erythrocyte destruction within the enlarged spleen has been postulated for many years as a probable mechanism of hæmolysis in cirrhosis (*e.g.*, by Eppinger, 1913; Jandl, 1955). But it is difficult to know the relative importance of this mechanism, particularly as splenectomy is seldom now undertaken in the presence of liver disease. Sheets and his co-workers (1951) reported, however, a return to a normal cell survival figure after splenectomy in one case and Jandl (1955) significant splenic sequestration of ^{51}Cr in an unspecified number of patients.

Pitcher and Williams (1963) obtained evidence for increased splenic hæmolysis in only two out of 10 patients suffering from miscellaneous types of liver disease, and Kimber, Deller, Ibbotson and Lander (1965), who carried out surface-counting studies on patients suffering from chronic liver disease, similarly found evidence of splenic sequestration of ^{51}Cr-tagged erythrocytes in only a small proportion of them, namely, in three out of 21. But, as already mentioned, they did find that the mean ^{51}Cr $T\frac{1}{2}$ was significantly lower in the patients whose spleens were palpable compared with those in whom the spleen could not be felt.

Holzbach, Shipley, Clark and Chudzik (1964) studied spleen size and the plasma clearance of heat-damaged ^{51}Cr-labelled erythrocytes, and also measured portal pressure: they found that the rate of removal of the heat-damaged cells could be correlated with the degree of splenomegaly but not with the severity of portal hypertension. They concluded that the mass of splenic tissue *per se* was important in relation to excess hæmolysis of heat-damaged erythrocytes in cirrhosis. They commented on the very limited evidence that is available relating spleen size to the survival of unheated erythrocytes.

Immunological Factors. Anti-erythrocyte antibodies are clearly an uncommon cause of hæmolysis in liver disease, as judged at least from the infrequency with which the direct antiglobulin test has been reported as positive.

The present author has no figures for the incidence of positive antiglobulin tests in cases of liver disease with or without increased hæmolysis, but of 250 cases of auto-immune hæmolytic anæmia of all types investigated only two patients, a child and an adult, had an underlying liver disorder, namely, chronic hepatitis and portal cirrhosis, respectively. The cause of auto-immunization in the few patients who do develop auto-antibodies is obscure. It is possible that these patients have an inborn proneness to develop auto-antibodies, *i.e.*, they are in any case candidates for auto-immune hæmolytic anæmia. Beickert and Siering (1958), in their case of immunopancytopenia, suggested that autolyzed liver tissue reaching the blood stream might have provided the antigen initiating the auto-immunization. It has to be admitted, however, that in the few instances of hæmolytic anæmia in which anti-erythrocyte auto-antibodies were shown to be present it has been far from evident that the antibodies were responsible for the excess hæmolysis.

Intrinsic Erythrocyte Defects. It is possible that metabolic defects of the erythrocytes may develop in some cases of liver disease. Pitcher and Williams (1963) reported, for instance, low reduced glutathione levels in 11 out of 26 patients studied, but the significance of this observation is not clear.

Liver Disease Complicating Established Hæmolytic Anæmia

Aside from hæmolysis developing as a complication of liver disease, there is the question of malfunction of the liver or actual structural damage developing as a sequel to established hæmolytic anæmia. It is, unfortunately, not always easy to be certain whether the liver is damaged to a significant degree in any particular case. Increased amounts of conjugated bilirubin in the serum are not uncommon in hæmolytic anæmia when the excretion of pigment is high (Tisdale, Klatskin and Kinsella, 1959) and its presence, in the absence of bile in the urine, cannot be taken as a clear sign of liver damage. Major amounts of bile in the urine do, however, probably denote actual liver-cell damage, and it is a fact that bilirubinuria, not apparently due to gallstones and biliary obstruction, has been reported from time to time in patients with severe grades of hæmolytic anæmia. In these patients it is probably the severity of the anæmia, and possibly autohæmagglutination, too, in some cases, which lead to anoxic damage to liver cells with consequential cloudy swelling, fatty degeneration and areas of focal necrosis (Lovibond, 1935; Farrar, Burnett and Steigman, 1940; Hyman and Southworth, 1951).

HÆMOLYTIC ANÆMIA IN RENAL FAILURE

Anæmia is a frequent accompaniment of renal failure. Its origin is in most cases the result of the interplay of a number of factors, of which hæmolysis is but one. In chronic renal disease, while it is true that the most severely anæmic patients are usually those with a severe degree of renal impairment, when a whole series of patients is compared the correlation between anæmia and azotæmia will be found not to be close (Roscoe, 1952), which is in accordance with the anæmia's complex origin. Anæmia, too, is a common accompaniment of acute renal failure, sometimes being severe and dramatic; and it is in these cases that hæmolysis often plays an important part in its causation.

Early Literature. Brown and Roth (1922) reviewed the pathogenesis of the anæmia of chronic nephritis and concluded that this was likely to be the result of the marrow being damaged along with the renal, retinal and cardiac tissues; they sought for evidence of increased hæmolysis but could not find any. Parsons and Ekola-Strolberg (1933) similarly concluded that in chronic nephritis the anæmia was due to depressed hæmopoiesis, and Nordenson (1938), Löwinger (1938) and Callen and Limarzi (1950) later came to the same conclusion.

Hæmolysis in Chronic Renal Failure

It was not until the Ashby method was used to study anæmic patients suffering from renal failure that evidence for a shortened erythrocyte life-span began to be obtained in individual patients, *e.g.*, by Emerson (1948), Emerson and Burrows (1949), Loge, Lange and Moore (1950), Bock, Böttner and Schlegel (1951–2) and Chaplin and Mollison (1953). Most of the patients in whom erythrocyte survival was impaired were in the terminal stages of chronic nephritis and were markedly anæmic and azotæmic. Loge, Lange and Moore (1950) demonstrated that the erythrocytes from a patient survived normally in a normal recipient and concluded that the hæmolytic mechanism was extracorpuscular in type.

Similar results were obtained after the introduction of radioactive chromium. Thus, Joske, McAlister and Prankerd (1956) found impaired survival in four out of 13 patients with chronic renal disease, the hæmolysis again appearing to be of the extracorpuscular type. Verel, Turnbull, Tudhope and Ross (1959) investigated 40 patients with "Bright's disease": six were found to have shortened erythrocyte survivals. Verel and his co-workers raised the question of a possible association between the onset of malignant hypertension and excessive hæmolysis; they also stressed that variations in total blood volume could make the ^{51}Cr survival data difficult to interpret in acutely ill patients. Borel and his co-workers (1961), too, found slight to moderate impairment of cell survival in four patients suffering from chronic pyelonephritis. The results of a study on 19 chronically uræmic patients have more recently been reported by Kurtides, Rambach, Alt and Del Greco (1964): the ^{51}Cr T$\frac{1}{2}$ was normal in 16 and slightly subnormal in three.

The reports referred to above do not contain as a rule much information on the blood pictures of the patients studied; but some of them were reported to have slightly or moderately raised reticulocyte counts. Loge, Lange and Moore (1950), however, also reported moderate increases in osmotic and mechanical fragilities in some cases, but without increase in serum bilirubin or fæcal urobilinogen. On the other hand, it seems generally to have been agreed that no specific or noteworthy changes in erythrocyte morphology are present in patients in whom there is no evidence for hæmolysis (Parsons and Ekola-Strolberg, 1933; Callen and Limarzi, 1950).

The data quoted above indicating some degree of increased hæmolysis were obtained from patients suffering from various types of chronic renal impairment; usually, however, they were in a progressive and terminal phase of their illness. It seems probable in fact that excess hæmolysis plays little or no part in the causation of the anæmia of patients suffering from stationary or only slowly progressive chronic renal disease. Under these circumstances anæmia seems to be the consequence wholly or almost wholly of impaired erythropoiesis. The finding by Muirhead and Shields (1954) of erythrophagocytosis and hæmosiderosis in the lymph nodes, spleen and liver after death in 20 of 21 patients who died of advanced bilateral renal disease is in accord with the concept that excess hæmolysis is not uncommon as a terminal event in such cases.

HÆMOLYSIS ASSOCIATED WITH ACUTE RENAL FAILURE: THE HÆMOLYTIC-URÆMIC SYNDROME

It is only in comparatively recent years that the association of overt hæmolysis with acute renal failure—the *hæmolytic-uræmic syndrome*—has been recognized. Now it is realized that this association in fact occurs quite frequently. Clinically, the syndrome occurs in three main forms: (1) an acute illness affecting infants or young children, now widely referred to as the "hæmolytic-uræmic syndrome", (2) an illness with more diverse clinical features, mainly affecting adults, and usually, too, acute in onset, generally referred to as thrombotic thrombocytopenic purpura, and (3) as a terminal feature in some cases of acutely progressive malignant hypertension or as an important manifestation of eclampsia or pre-eclampsia or of renal failure and hypertension occurring after childbirth.

The pathological basis for the renal failure varies, but focal glomerulonephritis, thrombotic microangiopathy, necrotizing arteriolitis and bilateral renal cortical necrosis are the commonest pathological processes found. In all the above conditions disease of small blood vessels is a conspicuous feature.

From the point of view of pathogenesis there are probably close analogies between the three main types of hæmolytic-uræmic syndrome. Each syndrome will, however, be discussed separately and these descriptions will be followed on pp. 881–85 by a further discussion of the blood picture—the "microangiopathic hæmolytic anæmia"—which is common to the hæmolytic-uræmic syndrome as a whole.

The distinctive feature of microangiopathic hæmolytic anæmia is the presence of numerous contracted, distorted and fragmenting erythrocytes in the peripheral blood (Figs. 207–10, etc.), and Brain, Dacie and Hourihane (1962) suggested that hæmolysis in this type of anæmia resulted in some way from a close and intimate contact between the erythrocytes and necrotic and often partially thrombosed small blood vessels through which they circulated (see p. 881).

THE HÆMOLYTIC-URÆMIC SYNDROME OF INFANCY AND CHILDHOOD

Early Literature. The first clear description of "hæmolytic anæmia in acute glomerulonephritis" seems to have been given by Hensley in 1952. The evidence for hæmolysis in each of three cases was quite impressive although the blood pictures were not described in detail. Thrombocytopenia (and severe anæmia) were both present in Hensley's third case, an infant, who died with severe oliguria on the 8th day of illness. At necropsy, the renal glomeruli and afferent arterioles were found to be affected by a severe necrotizing process. Wagner (1954) described another interesting case, but also unfortunately without giving details of the blood picture. This patient, a boy of 4 years, developed acute renal failure and anuria which was thought to be due to renal cortical necrosis. Severe anæmia was present and this was considered likely to be hæmolytic in type.

In the following year Gasser, Gautier, Steck, Siebenmann and Oechslin (1955) published their deservedly better known and widely quoted report under the title "Hämolytische-urämische Syndrome: bilaterale Nierenrindennekrosen bei akuten erworbenen hämolytischen Anämien". Five fatal cases were described in children, aged 2, 7, 13 and 14 months and 7 years, respectively. Their illnesses lasted from 7 to 30 days. At necropsy, bilateral renal cortical necrosis was found in all and in one of them the lesions of thrombotic microangiopathy as well. Thrombocytopenia was recorded in three. In one, the direct antiglobulin test was strongly positive. The blood film of one at least of the children was characterized by the presence of fragmenting erythrocytes ("zerfallende Formen") (Gasser *et al.*, Fig. 3).

The next publication of note was that of Fison (1956). Two successive siblings died when about 5 months of age of "acute glomerulonephritis" associated with severe anæmia for which repeated transfusions were required. The platelets were reported to be few in number and although details of the blood pictures are lacking there seems little doubt but that the anæmia was hæmolytic. At necropsy, glomerulitis with crescent formation was found and also fibrinoid necrosis in the afferent arterioles and hyaline thrombi in glomerular capillaries.

Further cases were described by Allison (1957) who stressed the presence of erythrocyte poikilocytosis and fragmentation. Of six children, four recovered. Post-mortem examination of the two who died showed platelet thrombi in the kidneys and other organs suggesting a diagnosis of thrombotic thrombocytopenic purpura. Aherne (1957) also briefly referred to two children who recovered—their blood films contained "burr" cells and "uræmic poikilocytes", and Robertson (1957) gave an account of two infants considered to have acute glomerulonephritis who developed concomitant acute hæmolytic anæmia. Small darkly stained erythrocytes "suggestive of spherocytes" were noted in their blood films: both these children recovered.

Other early reports include those of Mozziconacci and co-workers (1958), Peluffo, Temesio, Fernandez and Urtazum (1958), Debré and colleagues (1958), who stressed the association of thrombocytopenia with the nephropathy rather than the anæmia, and Habib, Mathieu and Royer (1958), who reviewed the clinical and hæmatological findings in 60 children who had died of renal disease. Of Habib, Mathieu and Royer's patients, six (aged 10 months to 11 years) had hæmolytic anæmia and it was noted that they had distorted and contracted erythrocytes in their blood films.

The whole subject of renal thrombotic microangiopathy in childhood was well reviewed by Royer, Habib and Mathieu (1960), who listed four types of case: (1) thrombotic microangiopathy, generalized but with renal manifestations (equated with Moschcowitz's disease (thrombotic thrombocytopenic purpura)); (2) thrombotic microangiopathy confined to the kidney; (3) renal cortical necrosis with hæmolytic anæmia and thrombocytopenic purpura, and (4) curable cases. The authors described eight personally-studied patients: one died of generalized thrombotic microangiopathy, four died with the kidney alone affected by the microangiopathy, two had fatal renal cortical necrosis and one child recovered. Royer, Habib and Mathieu stressed that the same bizarre blood picture was common to all four groups. Mozziconacci and his co-workers (1958), too, emphasized the link between contracted and distorted and triangular cells with renal syndromes in infants and thrombotic thrombocytopenic purpura.

More recently, accounts have been given by Lock and Dormandy (1961), under the heading "red-cell fragmentation syndrome" of five children, three of whom died, by Griffiths and Irving (1961) of three infants, all of whom recovered, by Gyselinck (1961) of one infant who recovered and by Javett and Senior (1962) of nine infants, seven of whom recovered.

Lamvik (1962) reviewed the case histories of 68 children who came to necropsy with the diagnosis of glomerulonephritis: only three were less than 1 year of age; but all three were severely anæmic, the result, it was thought, of excessive hæmolysis. The two tested were also thrombocytopenic.

Rosenthal, Schwartz, Yaffe and Kretchmer (1962) described briefly the occurrence of a syndrome of hæmolytic anæmia, thrombocytopenia and uræmia in seven children all under 3 years of age; in discussion it was agreed that the syndrome was not infrequent (in America) although it had up till then not been commonly reported.

Gianantonio and colleagues (1962) in a review of renal failure in infancy and childhood reported that 28 patients had had the hæmolytic-uræmic syndrome and that 13 of them had died. The disorder had appeared to occur in small epidemics. At necropsy in seven children, bilateral cortical necrosis was found in three and varying degrees of glomerular damage in four. The combination of severe anæmia with fragmenting erythrocytes and a negative direct antiglobulin test was recognized as the characteristic blood picture. Hæmoglobinæmia was observed in a few of the cases.

Brain (1963) recorded detailed clinical, hæmatological and necropsy findings in nine children who died of acute renal failure and "microangiopathic hæmolytic anæmia", as well as of one child who had a prolonged relapsing illness. The age of the nine children who died after acute illnesses varied from 4 months to 9 years; four had an upper respiratory tract infection which preceded their illness, and in four there was evidence of infection with a β-hæmolytic streptococcus; one child had diarrhœa and another otitis media. Their illnesses were ushered in by vomiting—a common symptom—œdema, purpura and/or pallor. Several of the children had hæmoglobinuria; seven had moderate to severe thrombocytopenia.

The pathology in Brain's cases is not easy to describe in brief but they all showed fibrinoid necrosis of the afferent gomerular arterioles and a greater or lesser degree of damage to glomeruli. In two of the patients hyaline thrombi were found in arterioles outside the kidney and these children may in fact have suffered from thrombotic thrombocytopenic purpura. In one patient there was histological evidence of renal cortical necrosis and in another of microscopic polyarteritis. Brain concluded by suggesting that the histological findings appeared to indicate a spectrum of possible vascular responses to a variety of factors, including streptococcal infection: in some patients, the vascular changes were strictly limited to the kidney but in others they were found in many organs and then gave rise to thrombotic thrombocytopenic purpura. In between a wide range of vascular responses could be found.

Recent Literature. In the last few years many more papers on the hæmolytic-uræmic syndrome have been published from many countries of the world, and it is now hardly an exaggeration to refer to the syndrome as a major pædiatric problem. Some of the more important papers are referred to below, after which the main clinical, hæmatological and pathological features of the syndrome will be summarized.

Noteworthy recent papers on the hæmolytic-uræmic syndrome include those of Bouissou, Fabre, Familiades and Regnier (1963), Giguens and co-workers (1963), Bartman, Jacques and Dustin (1964), Běluša (1964), Gaudier, Poingt, Lelong and Adenis (1964), Gianantonio and co-workers (1964), Shinton, Galpine, Kendall and Williams (1964), Shumway and Terplan (1964), Leikin (1964), Monnens and Retera (1965), Moorhead, Edwards and Goldsmith (1965), McQuiggan, Oliver, Littler and Cerny (1965), Guerra, Lorenzo y de Ibarreta and Temesio (1965), Barnard and Kibel (1965), Glasgow and Balduzzi (1965), Quoyle, Hirst and Painter (1965), Field and Kennedy (1965), McLean, Hilton Jones and Sutherland (1966), and Chaptel and co-workers (1966).

The paper of Bouissou and colleagues (1963) dealt primarily with the histopathology of the "nephroanæmias" of infancy: in fatal cases partial or total necrosis of glomeruli was to be expected, and sometimes this was associated with tubular necrosis. In infants who recovered the glomerular lesions were less severe but swelling and multiplication of endothelial cells approaching that seen in subacute glomerulonephritis could be expected. It was felt that all stages between minor focal glomerular lesions and cortical necrosis might be encountered. Bouissou and his colleagues concluded that the nephroanæmias of infancy were an entity distinct from Moschcowitz's disease (thrombotic thrombocytopenic purpura) and suggested that the similarity in blood picture could be explained if the causal mechanism of the anæmia was the same in the two conditions.

Bartman, Jacques and Dustin (1964) reported on an optical- and electron-microscopic study of renal biopsies, taken from an infant aged 6 months, 10 days after the start of his illness, and 18 months later. (References are given, too, to previous reports of renal biopsies.) The sections were considered to provide evidence for hæmolysis having occurred within pathologically altered glomeruli.

The report of Gianantonio and his co-workers (1964) is noteworthy for the large number of affected infants and children described: fiftyeight were observed in Buenos Aires between 1957 and 1963 and 17 further patients between May and December 1963. Their ages ranged from 4·5 to 36 months, the mean being 12·6 months; the mortality was 29%, and a virus ætiology was postulated. Shinton, Galpine, Kendall and Williams (1964) described 9 cases, seven in infants ranging in age from 6 weeks to 8 months. The histopathological features were carefully described. McQuiggan, Oliver, Littler and Cerny (1965) reported on six infants personally observed and reviewed the age distribution in a total of 60 cases: 39 were infants of 12 months of age or less.

Barnard and Kibel's (1965) account dealt with the occurrence of the hæmolytic-uræmic syndrome in central Africa: 11 local cases were described, nine being European infants. Barnard and Kibel emphasized the similarity in clinical picture from case to case—the onset with diarrhœa, then vomiting, then pallor and oliguria. Their histological studies suggested that hæmolysis took place within glomerular capillaries and they pointed out that distorted erythrocytes might be found in the urinary deposit as well as being a characteristic feature in peripheral blood films.

Chaptal and co-workers (1966) described four infants all of whom recovered and in whom serial renal biopsies were carried out. The glomerular lesions, although typical of thrombotic microangiopathy, were less severe and fewer in number than in rapidly fatal cases; the affected glomeruli eventually fibrosed within a period of several months.

McLean, Hilton Jones and Sutherland (1966) reported on 10 cases occurring within a small geographical area within a 5-months' period—nine of the cases occurred in July–August 1963. No infective agent was, however, isolated. One child died of renal cortical necrosis, the others, aged 6 weeks to 3½ years, recovered. The series is perhaps unusual in that none of the children had platelet counts less than 100,000 per cu. mm.

The Hæmolytic-Uræmic Syndrome of Infancy and Childhood: A Summary

The main *clinical features* of this important disorder, which is being reported with increasing frequency from most countries of the world, are as follows: infants and young children of both sexes are affected, particularly those between 5 and 12 months of age. Gastro-intestinal symptoms—diarrhœa followed by vomiting—have been the presenting feature in most cases and pyrexia is often present also. Within 5–14 days as a rule—often when the abdominal symptoms have subsided—pallor develops and sometimes slight jaundice and often, too, oliguria or even anuria. The urine passed is dark in colour and contains urobilin, free red cells and sometimes free hæmoglobin. Hæmorrhages from mucous membranes may occur, as well as bruising and skin petechiæ. The spleen is often palpable. Symptoms and signs of uræmia and cardiac failure may develop later, but evidence of nervous system involvement—so characteristic of thrombotic thrombocytopenic purpura—is usually lacking.

The blood picture is distinctive. Anæmia is often severe, and this is accompanied by a neutrophil leucocytosis and a variable degree of thrombocytopenia, which may be marked. Stained films show a characteristic microangiopathic hæmolytic anæmia picture, with contracted, distorted and fragmented erythrocytes, occasional erythroblasts and a raised reticulocyte count (see p. 882 and Figs. 207–210 etc.). The direct antiglobulin test typically is negative. The serum-bilirubin level is elevated but does not usually exceed 1–2 mg. per 100 ml. The degree of azotæmia varies from case to case but in the presence of anuria the blood urea often reaches 300 mg. per 100 ml. or more.

Treatment and Prognosis

The outlook is serious but not hopeless; and although the mortality in most of the reported large series has exceeded 50% that of Gianantonio and co-workers (1964) was only 29%. A good deal depends perhaps upon experience and skill in the treatment of uræmic infants, as well, of course, on the extent and severity of the kidney lesions. Peritoneal dialysis has been advocated as a valuable means of palliative therapy (Barnard and Kibel, 1965; McQuiggan *et al.*, 1965; Moorhead, Edwards and Goldsmith, 1965; Troelstra and Visser, 1965). Steroids have often been used in treatment but little if any benefit results; splenectomy should probably not be attempted.

Heparin Therapy. Heparin has been tried on a few occasions but its possibilities have probably not yet been fully explored.

Hitzig (1964) described the results of treating with heparin four infants or young children who had the hæmolytic-uræmic syndrome and one infant who was considered to have a more generalized microangiopathy. Four of the children recovered and in three of them at least the heparin therapy appeared to be the turning point in their illness.

Kibel and Barnard (1964) and Barnard and Kibel (1965) reported full recovery after 4 days of anuria in an infant given 1,000 i.u. of heparin b.d. for 5 days, and another apparently favourable result was reported by Künzer and Aalam (1964a, b) in a 12-year-old boy. Another apparently favourable response, leading to complete recovery, was reported by Desmit and co-workers (1965). Some of the data in this infant's case were suggestive of a hypercoagulable state. Two days after the start of heparin therapy a diuresis occurred (following 5 days of anuria) while the fibrinogen and platelet count returned to normal in 4–6 days. (The possible value of heparin is discussed further on p. 873 when the treatment of thrombotic thrombocytopenic purpura is discussed. See also Case 29, p. 850.)

Pathology

The main lesions are to be found in the renal glomeruli, some or all of which may be affected. The changes include: endothelial swelling and proliferation, engorgement with blood and thickening of the basement membrane affecting the whole or part of a glomerulus; exudate into the capsular space and sometimes adhesions and crescent formation; thrombi within glomerular capillaries as a regular feature and sometimes necrosis of whole or part of a glomerulus. Fibrinoid necrosis and intraluminal thrombi may be seen in afferent arterioles also, and in the worst affected cases patchy or extensive cortical necrosis. Protein and blood casts are usually present in some of the tubules.

Vascular lesions—fibrinoid necrosis of arterioles and hyaline thrombi in capillaries—may be found in other organs but these changes do not seem to be commonly met with (see p. 859, under *Thrombotic Thrombocytopenic Purpura in Childhood*).

Aetiology. The cause of the hæmolytic-uræmic syndrome is not yet fully understood. A hypersensitivity (allergic) mechanism has been suspected but not proved (McQuiggan *et al.*, 1965), and analogies have been drawn between the human disease and the Shwartzman reaction in experimental animals following injections of bacterial endotoxin (Shwartzman, Klemperer and Gerber, 1936; Shumway and Terplan, 1964; Barnard and Kibel, 1965). It is particularly interesting to note that the syndrome has occurred on several occasions shortly after vaccination against smallpox (Monnens and Retera, 1965) or injections of "triple" vaccine (Peluffo *et al.*, 1958; Guerra *et al.*, 1965; Quole, Hirst and Painter, 1965).

Virus infection has been suspected as the initiating cause and most authors have stressed the occurrence of preceding gastroenteritis. Although viruses have in fact been sought by several groups of workers the results have mostly been negative (Gaudier *et al.*, 1964; Bartman, Jacques and Dustin, 1964; Barnard and Kibel, 1965; McLean, Hilton Jones and Sutherland, 1966). Some evidence in favour of a virus infection—isolation from the blood in five children and complement-fixing antibodies present in fifteen—was, however, obtained by Gianantonio and co-workers (1964) in infants studied in Argentine. Gianantonio also demonstrated a sharp decrease in γG-globulin levels at the onset of their illness in 11 children and suggested that the globulin might have been deposited in the vascular bed in response to an immunological stimulus. A

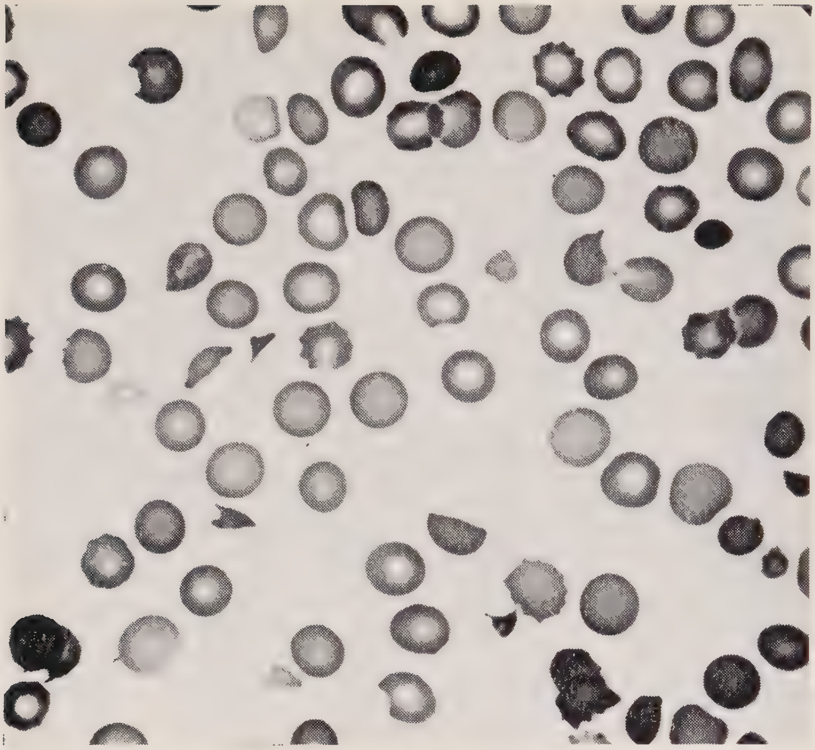

FIG. 207. Photomicrograph of the blood film of a boy aged 8 years who died of the hæmolytic-uræmic syndrome (Case 27).

There is marked anisocytosis and many contracted erythrocytes and irregularly shaped cell fragments, including triangular forms, are present. × 700.

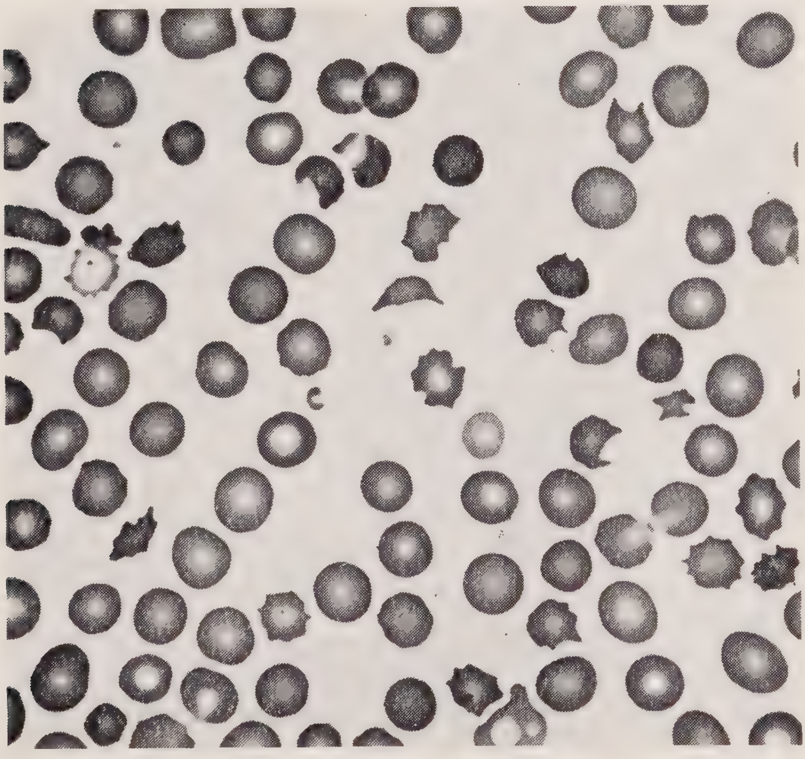

FIG. 208. Photomicrograph of the blood film of a 6-months-old infant who died of the hæmolytic-uræmic syndrome (Case 28). There is marked anisocytosis, a tendency to spherocytosis and considerable crenation. Irregularly shaped cells, "burr" cells and cell fragments are conspicuous. × 700.

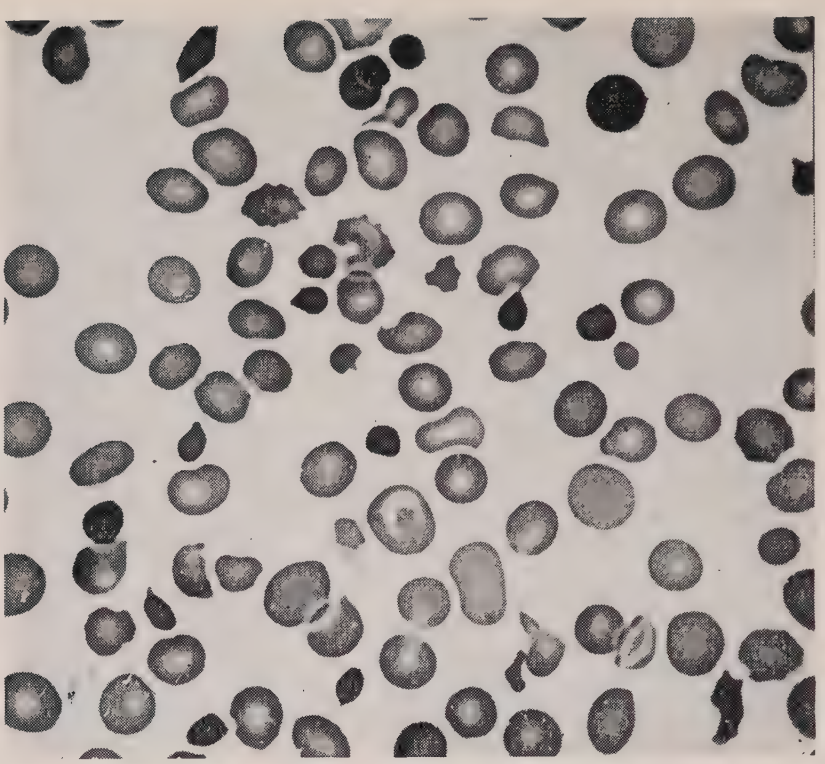

FIG. 209. Photomicrograph of the blood film of a boy aged 2½ years suffering from the hæmolytic-uræmic syndrome (Case 29). There is marked anisocytosis and many small contracted cells and cell fragments are present as well as some macrocytes. Most of the contracted cells and cell fragments are irregular in contour but a few perfectly round spherocytes can also be seen. ×700.

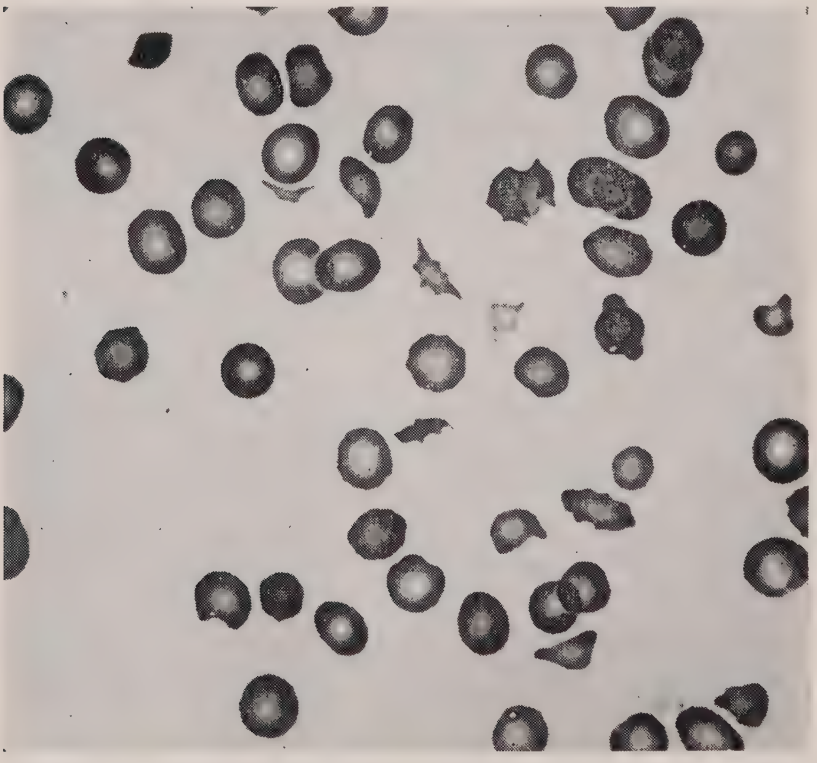

FIG. 210. Photomicrograph of the blood film of a boy aged 9 who suffered from the hæmolytic-uræmic syndrome (Case 30). There is considerable anisocytosis and quite large numbers of irregularly contracted cells and cell fragments are present, including some "burr" cells. ×700.

Coxsackie virus (group A, type 4) was isolated from a mild case by Glasgow and Balduzzi (1965).

The histories of four personally observed patients are described briefly in the following case reports. In one child heparin therapy appeared to be the turning point in his illness (Case 29).

Case Report: Fatal Hæmolytic-Uræmic Syndrome in a Child; Microangiopathic Hæmolytic Anæmia

Case 27. The patient, T. A., a boy aged 8 years, developed a head cold on the 23rd December 1960; 4 days later his face was noticed to have become swollen and he had a transient rash on his knees. One week after the start of his illness a further rash (? petechial) was noticed

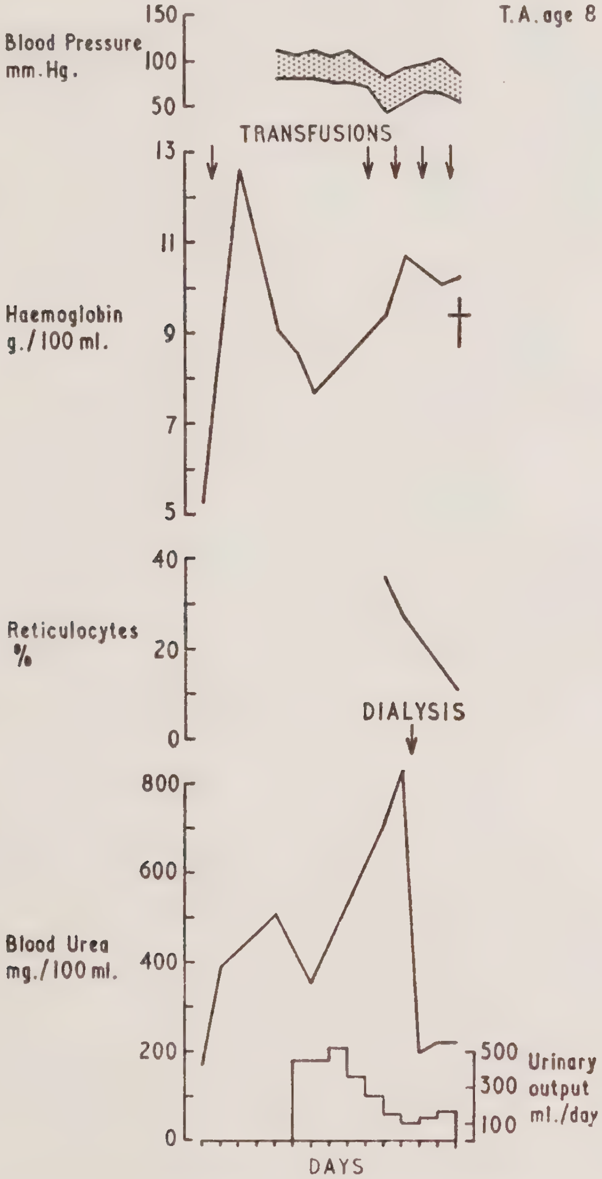

FIG. 211. Diagram illustrating the clinical course of, and laboratory findings in, a boy who died of the hæmolytic-uræmic syndrome (Case 27).

on his neck and trunk and he started to vomit. This led to his admission to hospital where he was found to be anæmic and uræmic: the hæmoglobin was 5·3 g. per 100 ml., reticulocyte count 7% and serum bilirubin 2·5 mg. per 100 ml.; the blood urea was 178 mg. per 100 ml. He was transfused and treated with adrenocorticosteroids, but his general condition, nevertheless, deteriorated and he had epistaxes, and because of a rise in blood urea and a falling urinary output he was transferred to Hammersmith Hospital for possible hæmodialysis on the 13th January 1961.

Physical Examination. On admission, he was found to be pale and sallow in colour, and generally very ill and severely dehydrated. His systolic blood pressure was 50 mm. Hg but on correction of dehydration this rose to 90/55. He had neither papillœdema nor retinopathy.

Laboratory Findings. The hæmoglobin on admission was 9·4 g. per 100 ml., reticulocyte count 36%, leucocyte count 16,000 per cu. mm., with 76% neutrophils and 6% metamyelocytes, and platelet count 172,000 per cu. mm. Stained films showed a well marked microangiopathic hæmolytic anæmia picture: there was marked anisocytosis and polychromasia and normoblasts were conspicuous (1,600 per cu. mm.). A striking feature was the presence of numerous contracted and distorted erythrocytes and cell fragments, some triangular in shape; small numbers of rounded microspherocytes could also be seen (Fig. 207). The direct antiglobulin test was negative. The blood urea was 720 mg. per 100 ml. The urine contained protein and erythrocytes were present in the deposit.

Erythrocyte osmotic fragility was increased, lysis commencing in 0·80% NaCl and there was 40% lysis in 0·50% NaCl.

Further Progress (Fig. 211). He was transfused on several occasions, but despite dialysis which substantially reduced the blood urea, his general condition further deteriorated and with a falling urinary output he died on the 18th January 1961 after an illness lasting between 3 and 4 weeks. At no time was his blood pressure elevated.

Necropsy. The main abnormalities were found in the kidneys. Histological examination showed widespread fibrinoid necrosis of arterioles and hæmorrhagic infarction of many of the glomeruli; others showed lobular proliferation and occasional capsular adhesions. A few neutrophil leucocytes were seen in infarcted or partially infarcted glomeruli. The intrarenal arteries appeared normal. Hæmosiderin was present in some renal tubular cells.

The spleen contained an excess of iron; a small thrombus was found in a single arteriole in the myocardium.

Summary. A fatal case of the hæmolytic-uræmic syndrome in a boy. The main pathological features were fibrinoid necrosis of the renal arterioles and infarction of glomeruli. Death resulted from renal failure.

Case Report: Hæmolytic-Uræmic Syndrome in an Infant: Renal Cortical Necrosis and Microangiopathic Hæmolytic Anæmia

Case 28. The patient, G. Y., was a 6-months-old male infant. One month before admission to hospital he had had an attack of otitis media which responded well to treatment with penicillin. The day before admission he vomited and had diarrhœa. When examined he was found to be restless and slightly febrile, but except for pallor there were no abnormal physical signs; the blood pressure was, however, 130/70.

Laboratory studies showed that he was anæmic and also uræmic: the hæmoglobin was 6·2 g. per 100 ml., leucocyte count 10,600 per cu. mm., reticulocyte count 1·6%, serum bilirubin 2·4 mg. per 100 ml. and blood urea 242 mg. per 100 ml. The direct antiglobulin test was negative and the antistreptolysin titre 50 units. The urine contained protein, and erythrocytes and granular casts were seen in the deposit.

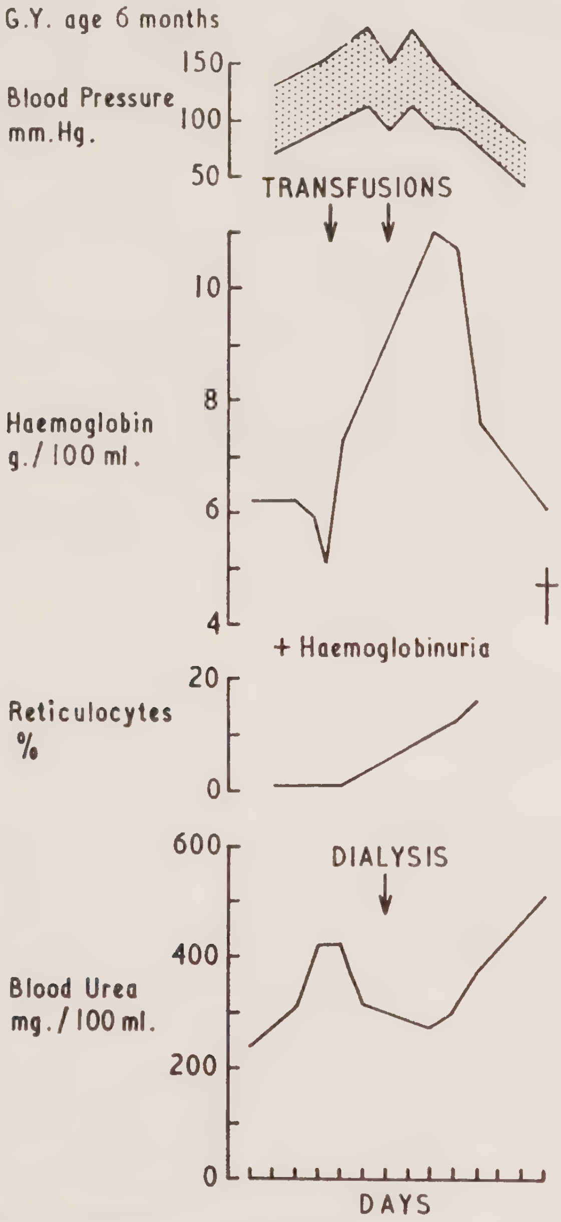

FIG. 212. Diagram illustrating the clinical course of, and laboratory findings in, an infant who died of the hæmolytic-uræmic syndrome (Case 28).

He had a convulsion and became anuric and was transferred to Hammersmith Hospital on the 24th July 1959, 4 days after the start of his illness.

On admission, he was found to be pale but to be free from purpura or œdema. The blood pressure was 150/90.

Laboratory Findings. The hæmoglobin was 5·1 g. per 100 ml., leucocyte count 37,000 per cu. mm., with 55% neutrophils, and platelet count 85,000 per cu. mm. The reticulocyte count was relatively low at first (1·9%) but rose to 16·4% subsequently. Stained films showed a microangiopathic hæmolytic anæmia picture: there was a considerable degree of anisocytosis and polychromasia and some spherocytosis and also moderate numbers of bizarre-shaped contracted and distorted cells and cell fragments (Fig. 208).

Further Progress. He was transfused and dialysed but the hæmoglobin fell rapidly and the blood urea rose again to more than 400 mg. per 100 ml. (Fig. 212). His general condition deteriorated, too, and he remained virtually anuric. He died on the 4th August 1959 after an acute illness of just over 2 weeks' duration.

Necropsy Findings. The important pathological findings were confined to the kidneys, which macroscopically were mottled red and yellow in colour. Sections revealed patchy bilateral cortical necrosis; moreover, in the areas of surviving renal tissue many of the glomeruli were seen to be partially hyalinized in association with a variable degree of fibrinoid necrosis of the afferent arterioles.

Summary. The patient, an infant, died of renal failure consequent on bilateral renal cortical necrosis. His short illness was accompanied by a severe microangiopathic hæmolytic anæmia.

Case Report: Hæmolytic-Uræmic Syndrome in a Child: Microangiopathic Hæmolytic Anæmia; Recovery

Case 29. P. W., a boy aged 2½ years, was quite well until the 10th June 1963 when he complained of abdominal pain and diarrhœa. The next day blood was passed with loose stools and he vomited several times. The diarrhœa and vomiting continued over the following 2 days and he became progressively more lethargic and pale. He was admitted to the Radcliffe Infirmary, Oxford, under the care of Dr. H. L. Ellis on the 18th June when his hæmoglobin was found to have fallen to 5·3 g. per 100 ml.; the reticulocyte count was raised (15%) and the platelet count was subnormal (60,000 per cu. mm.). He was transfused twice with 500 ml. of packed erythrocytes and given 10 mg. of prednisone 8-hourly. The following day he was found to be oliguric; the blood urea was 325 mg. per 100 ml. He was then given 1,000 i.u. of heparin intravenously and this was followed by further heparin given continuously intravenously at a dose-rate of 2,000 units 2-hourly together with 400 ml. of Rheomacrodex per 24 hours. During the night he passed 150 ml. of urine but by the next day (21st June) his blood urea was found to have risen to 520 mg. per 100 ml. In view of the possible necessity for hæmodialysis he was transferred to Hammersmith Hospital.

Physical Examination. On admission he was found to be pale and restless and there were bruises at the sites of venepuncture but no purpura or œdema. The spleen was not palpable but the liver could just be felt. The blood pressure was 130/? mm. Hg. He had melæna and the urine appeared blood-stained; it contained protein and many erythrocytes were seen microscopically.

Laboratory Findings. On admission to Hammersmith Hospital, on the 21st June 1963, the hæmoglobin was 10·2 g. per 100 ml.; the level fell continuously over the next few days and by the 27th June it had reached a value of 4·4 g. per 100 ml., at which point he was transfused (see Fig. 213). The platelet count on admission was 60,000 per cu. mm. and this fell to a minimum of 36,000 per cu. mm. on the 26th June. The leucocyte count at no time exceeded 9,000 per cu. mm., with 69% neutrophils. The serum bilirubin was 0·6 mg. per 100 ml. on the 27th June.

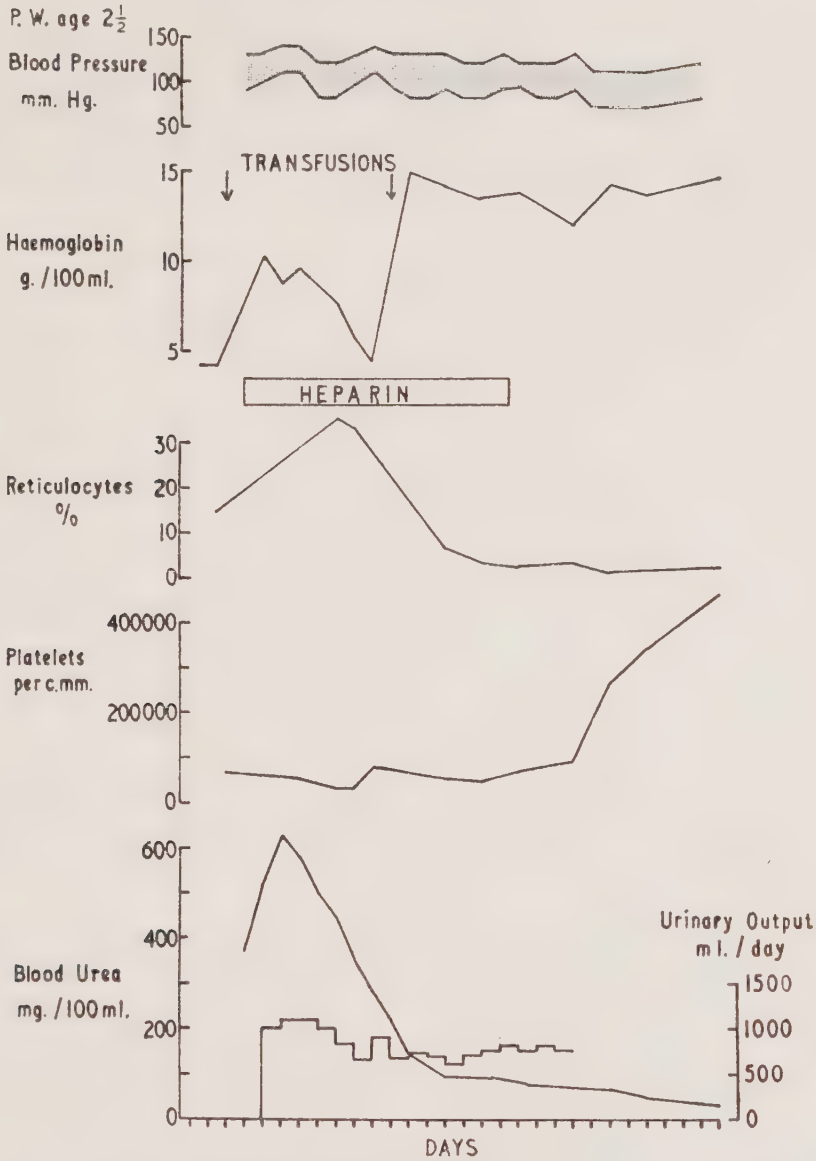

FIG. 213. Diagram illustrating the clinical course of, and laboratory findings in, a boy who recovered from the hæmolytic-uræmic syndrome (Case 29).

Blood films showed a typical microangiopathic picture (Fig. 209), and numerous bizarre-shaped contracted cells and cell fragments were present, in addition to marked anisocytosis and polychromasia.

The blood urea was 625 mg. per 100 ml. on admission (Fig. 213) but it subsequently fell steadily. The output of urine was found to be reasonably good on admission and this was maintained for the next 3 weeks at about 1,000 ml. daily or a little under.

Treatment and Progress. Intravenous heparin therapy was continued but, in view of the apparently recovering renal function, the dose was drastically reduced on the 22nd June to 250 i.u. every 4 hours. This regime was kept up until the 4th July on which day intravenous fluid therapy was finally discontinued. On the 22nd June the heparin was given in 500 ml. of Rheomacrodex; subsequently, it was given dissolved in isotonic saline.

Clinical improvement leading to complete recovery followed the restoration to normal of the blood picture and renal function.

Summary. A small boy suffered from the hæmolytic-uræmic syndrome which was ushered in by gastrointestinal symptoms. Recovery took place and this may have been helped by, or even been initiated by, heparin therapy.

Case Report: Microangiopathic Hæmolytic Anæmia Associated with Microscopic Polyarteritis Nodosa

Case 30. The patient, D. W., was a boy aged 9 years. In mid-December 1959 he developed a sore throat. Two weeks after this he had a headache and vomited, and a few days later he complained of malaise and pain in the loins, vomited again and had diarrhœa. His face was noticed to be œdematous and the urine was found to contain blood and protein. He was admitted to hospital where the blood pressure was found to be 160/110, hæmoglobin 12·1 g. per 100 ml. and blood urea 146 mg. per 100 ml. The urine deposit contained many erythrocytes as well as leucocytes and granular casts; the serum antistreptolysin titre was markedly elevated (800 units per ml.).

His progress is illustrated in Fig. 214. He became more and more anæmic and by the 8th February 1960 the hæmoglobin had fallen to 5·2 g. per 100 ml. He also had a continuous leucocytosis and neutrophilia, the highest recorded count being 77,000 per cu. mm. with more than 90% neutrophils.

His general condition began to deteriorate and on the 15th February 1960 he was transferred to Hammersmith Hospital for renal dialysis.

On admission, he was found to be very ill and although conscious he was drowsy. He was very pale and there were signs of fading purpura on the trunk. The blood pressure was 155/105. The hæmoglobin was 3·3 g. per 100 ml., leucocyte count 37,000 per cu. mm., platelet count 69,000 per cu. mm. and blood urea 580 mg. per 100 ml.

Stained films showed the picture of microangiopathic hæmolytic anæmia: there was a moderate degree of anisocytosis and polychromasia and some contracted and distorted erythrocytes and cell fragments were present (Fig. 210).

Further Progress (Fig. 214). He was dialysed and transfused, as a result of which blood urea was substantially reduced and the hæmoglobin raised. Anæmia, however, rapidly recurred and the blood urea rose steeply, too. He became oliguric and his blood pressure rose to 180/140 before his death on the 23rd February 1960.

Necropsy Findings. Both kidneys appeared slightly swollen and showed fine petechial hæmorrhages over their surface. Sections of the kidneys showed areas of fibrinoid necrosis which affected all coats of the walls of interlobular arteries and an intense polymorphonuclear reaction was present in the surrounding periarterial tissue. Some of the glomeruli were totally necrotic and infiltrated with neutrophils; others were infarcted; the majority showed some endothelial proliferation. Similar vascular lesions were seen in arterioles in the adrenals, pancreas and myocardium; in each instance there was destruction and fibrinoid necrosis throughout the whole wall of the affected vessel as well as a characteristic periarteriolar inflammatory reaction.

Summary. A young boy died of an acute illness simulating acute glomerulonephritis. Necropsy, however, revealed the histological changes of microscopic polyarteritis nodosa. Progression of his illness was associated with a well marked microangiopathic hæmolytic anæmia.

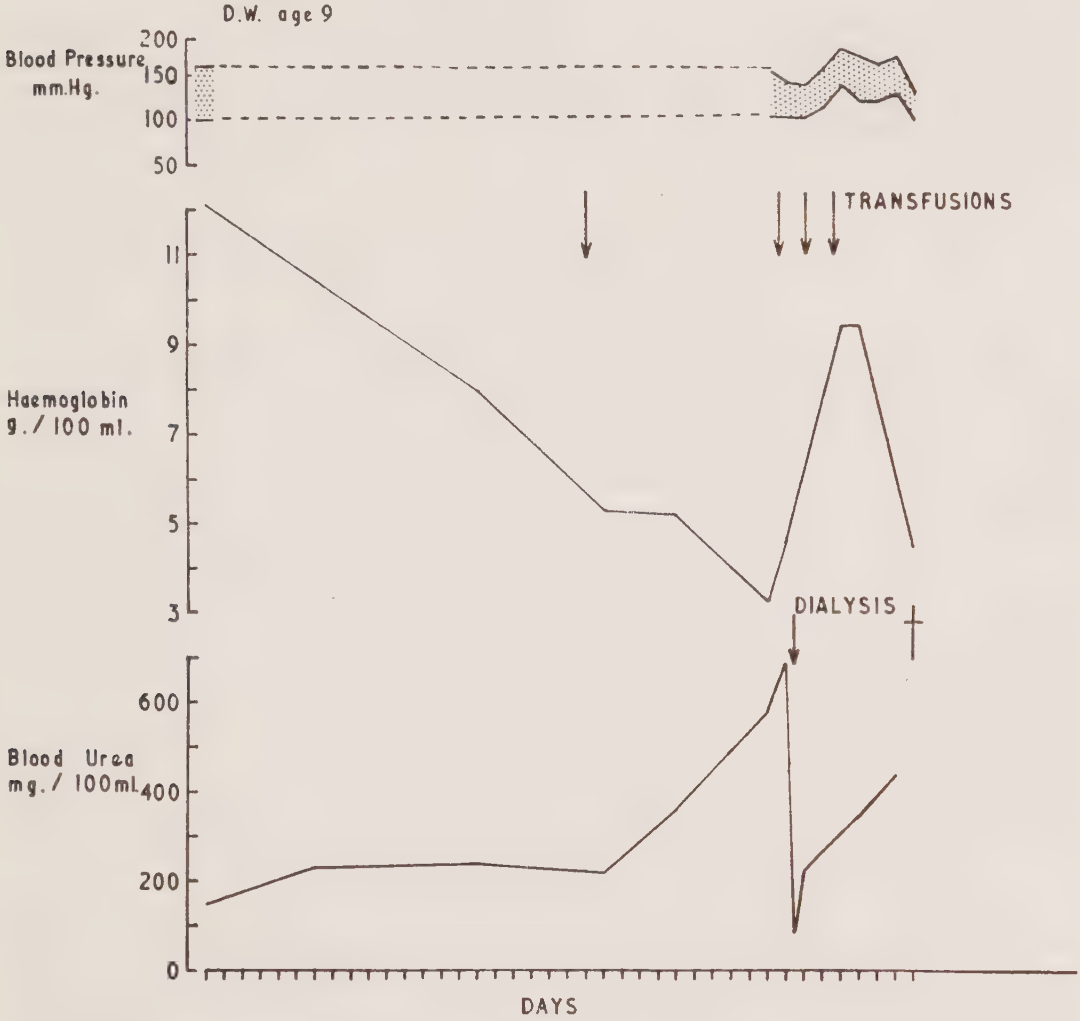

FIG. 214. Diagram illustrating the clinical course of, and the laboratory findings in, a boy who died of the hæmolytic-uræmic syndrome (Case 30).

Acute Hæmolytic Anæmia Associated with Renal Insufficiency in the Newborn

A few cases have been described, of obscure origin, in which acute hæmolytic anæmia associated with azotæmia has developed within the first week of life. Mégevand, Wyss and Thélin (1956) described the case of an infant who became jaundiced on the 3rd day and who died when 6 days of age. At necropsy there was kernicterus and the kidneys showed ischæmia of the cortices and hæmorrhagic infarction of renal papillæ. A further infant who presented with a similar clinical story was described by Massimo and Rainero (1963): this infant, however, recovered after treatment with prednisone, intravenous albumin and blood transfusion. A rare type of renal thrombotic microangiopathy was postulated.

The Hæmolytic-Uræmic Syndrome in Radiation Nephritis

A rare cause of the hæmolytic-uræmic syndrome is radiation nephritis. Beck (1958) described the occurrence of severe anæmia, with "burr" cells and polychromasia in blood films, and severe azotæmia in a child who had received abdominal irradiation for a Wilms's tumour. Histological examination of the kidneys showed fibrinoid and hæmorrhagic necrosis of glomerular tufts and fibrinoid necrosis of the walls of many of the arterioles.

THE HÆMOLYTIC-URÆMIC SYNDROME IN ADULTS

There is no question but that the hæmolytic-uræmic syndrome is more common in children than in adults and that infants are particularly liable to be affected. In adults the syndrome takes a different form, although the blood picture is the same. Clinically the patients usually present with the symptoms and signs either of (I) thrombotic thrombocytopenic purpura or of (II) acutely progressive malignant hypertension, often with oliguria, or eclampsia or pre-eclampsia or hypertension and renal failure *post partum.* In all the above situations fibrinoid necrosis of arterioles and intraluminal hyaline thrombi are present.

I. THROMBOTIC THROMBOCYTOPENIC PURPURA (MOSCHCOWITZ'S DISEASE)

Thrombotic thrombocytopenic purpura (TTP) is now widely recognized as an important and serious but uncommon disorder characterized by hæmolytic anæmia, purpura, renal impairment, neurological signs and often pyrexia. Adults of both sexes are mainly affected. The disease, however, probably occurs also in childhood, and it then merges clinically and pathologically into the hæmolytic-uræmic syndrome of infancy and childhood which has been described above.

Its basis seems to be widespread disease of small blood vessels (arterioles and arteriolar capillaries). The first case was described by Moschcowitz in 1924 and 1925.

In the following descriptions the hæmolytic side of the disease receives the most attention.

Synonyms. Thrombotic thrombocytopenic purpura (Singer, Bornstein and Wile, 1947); thrombocytic acroangiothrombosis (Fitzgerald, Auerbach and Frame, 1947), Moschcowitz's syndrome (Lennox and Dacie, 1951); platelet thrombosis syndrome (Symmers and Barrowcliff, 1961; Beigelman, 1951); thrombotic microangiopathic hæmolytic anæmia (thrombotic microangiopathy) (Symmers, 1952); thrombohemolytic thrombocytopenic purpura (Adelson, Heitzman and Fennessey, 1954).

Early Accounts in the Literature. Moschcowitz (1924, 1925) described under the title "An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries. An undescribed disease" the case of a young female, aged 16, who was admitted into hospital severely anæmic after a 10 days' illness. She was febrile, and petechiæ were seen on her left arm. No platelet count was performed. She died soon afterwards and at necropsy widespread hyaline thrombi were found in small blood vessels. Moschcowitz concluded that the patient's disease might be based on "some powerful poison which had both agglutination and hemolytic properties" and considered that the hyaline thrombi could have been derived from agglutinated erythrocytes. Osmotic fragility was recorded as being notably increased and there is little doubt but that Moschcowitz thought that his patient's anæmia was hæmolytic in origin.

The next cases were described by Baehr, Klemperer and Schifrin (1936). They were aware of Moschcowitz's report and described four female patients, all of whom were severely anæmic and had raised reticulocyte counts. They were markedly thrombocytopenic, too, and had severe purpura. Three of the patients were slightly icteric. At necropsy, exactly the same pathological lesions were found as in Moschcowitz's case, but the thrombi were considered mainly to consist of agglutinated platelets. Baehr and his colleagues considered that the anæmia was in all probability due to hæmorrhage into the tissues and that the consequent extravascular destruction of the erythrocytes was responsible for the icterus.

Baehr, Klemperer and Schifrin's Case I was also reported in more detail by Friedberg and Gross (1936) as a case of "nonbacterial thrombotic endocarditis associated with acute thrombocytopenic purpura." Friedberg and Gross remarked on the failure of transfusions to affect the severity of the anæmia and on the icterus and the raised urinary urobilin excretion and concluded that the erythrocytes were being rapidly destroyed. A further probable example was described by Gitlow and Goldmark (1939, their Case I) under the title "generalized capillary and arteriolar thrombosis". Their patient, a girl aged 18 (observed in 1932), was markedly anæmic; she had a high reticulocyte count (31–80%) and moderate thrombocytopenia (50,000–100,000 platelets per cu. mm.). She died in coma and at necropsy thrombi were found in the small blood vessels of many organs.

Altschule (1942) described a further case. Again, severe anæmia was accompanied by a raised reticulocyte count (12%) and there was severe thrombocytopenia. The thrombi were considered to be formed largely of platelets deposited upon damaged endothelium. An analogy was drawn with the Schwartzman (*sic*) phenomenon. Another case was described by Bernheim (1943–4). This account is of particular interest as the patient's brother was reported to have died of periarteritis nodosa.

A further case was described by Trobaugh, Markowitz and Davidson (1946). This paper seems to be the first in which hæmolytic anæmia was mentioned in its title. These authors, too, were satisfied that the condition they were studying was quite distinct from Werlhof's thrombocytopenic purpura and that the anæmia was truly hæmolytic in origin and not due to loss of blood by extraversation. Like Altschule, they drew attention to the similarity of the vascular lesions to those of the Shwartzman reaction and agreed, too, with Altschule, that the platelets could have been deposited upon damaged endothelium.

Upon the basis of these early reports a large literature has been built up. It is impossible to list this in its entirety; however, in the following selected papers are described cases in which hæmolytic anæmia was an important feature or in which this aspect of the patient's illness was particularly well investigated or documented: Muirhead, Crass and Hill (1948); Singer, Motulsky and Shanberge (1950); Goldenberg, Thayer and Hastings (1950); Meacham, Orbison, Heinle, Steele and Schaefer (1951); Adelson and Stefanini (1952); Adelson, Heitzman and Fennessey (1954); Symmers (1956a); Ritz, Groisser and Banowitch (1956); Báez-Villaseñor and Ambrosius (1957); Shumway and Miller (1957); Brittingham and Chaplin (1957); Epstein (1957); Rubinstein, Kagan, MacGillviray, Merliss and Sacks (1959); Bernstock and Hirson (1960); van Gool and Sluiter (1960); Sansone, Buffoni and Rosso (1961); MacWhinney, Packer, Miller and Greendyke (1962); Swaiman, Schaffhausen and Krivit (1962); McCormack, O'Brien and Oliver (1963). Certain other atypical cases are referred to later (p. 858).

Comprehensive reviews, usually including one or more case reports, include those of Singer, Bornstein and Wile (1947), Rackow, Steingold and Wood (1952), Symmers (1952), Singer (1954), Wasserman (1958), Antes (1958), Cahalane and Horn (1959), Levine and Shearn (1964) and Amorosi and Ultmann (1966).

Clinical Features

Typically, thrombotic thrombocytopenic purpura (TTP) runs a short, acute and fatal course. Of 116 patients reviewed by

Cahalane and Horn (1959), 98 died within a few weeks. The patients present with anæmia, a variable degree of icterus, skin purpura and perhaps bleeding from the mucous membranes, and often symptoms of uræmia; occasionally anuria may develop. Pyrexia is usually present also. In addition, variable and varying neurological signs are likely to be present; these range from transient pareses to deep coma. As already mentioned, adults of all ages are chiefly affected and the disease seems to be more frequent in females—according to Amorosi and Ultmann (1966) in a ratio of 3:2.

The patients' signs and symptoms generally intensify as the days pass, attempts at treatment are usually unavailing and death follows, as a rule from hæmorrhage or renal failure.

The above brief description may be said to refer to the disease in its typical and most malignant form; then the symptoms and laboratory signs together make an unmistakable picture. However, less typical cases occur and difficulty in diagnosis may then arise. The disease may run a chronic and fluctuating course; the neurological signs (or renal signs) may be minimal; purpura, too, may be inconspicuous or absent. Furthermore, recovery occasionally takes place, either spontaneously or induced apparently by treatment.

The pathology of the disease may vary also. Whereas typically the vascular lesions are widespread and affect small arterioles and arteriolar capillaries in many organs and tissues, occasionally they may be more localized and affect perhaps the kidney or brain predominantly—with corresponding localization of symptoms and signs. In some cases, too, features suggestive of systemic lupus erythematosus may be present also (see below), and in other instances where the kidney is predominantly or apparently solely affected the vascular changes may be difficult to distinguish from those of focal glomerulonephritis. In yet other cases the lesions in small blood vessels are accompanied by thrombophlebitis and thrombosis in larger vessels and macroscopic infarcts. Vegetations on the mitral valves or verrucous endocarditis (no organisms detectable) have also been observed (Friedberg and Gross, 1936; Meacham *et al.*, 1951; Gendel, Young and Kraus, 1952; Levine and Shearn, 1964).

Some of the disease's more atypical clinical manifestations are dealt with briefly below:

Chronic and Atypical Cases

Meacham and co-workers (1951) described a patient whose illness lasted for 3 years and in whom a 2-year remission followed splenectomy.

Beigelman (1951) reported on two patients: the first had a 10-year history of arthralgia, angina-like attacks and mild anæmia and died after a terminal illness lasting about 4 months; the second died after an illness lasting about 2¾ years. In both cases pathological features suggestive of disseminated lupus were thought to be present in addition to signs of TTP.

Dacie and his colleagues (1953) described the case of a young girl who died when almost 7 years of age after an illness of at least 3 years' duration characterized by thrombocytopenic purpura, hæmolytic anæmia and terminally by renal failure and malignant hypertension. Splenectomy had been carried out early in the course of the illness but it had had no effect. The blood film was strikingly abnormal and typical of a severe microangiopathic hæmolytic anæmia (Fig. 216). At necropsy, fibrin-containing thrombi were found in the brain, myocardium, œsophagus, duodenum and kidney, the latter being diffusely involved. The presence of fibrosed glomeruli was compatible with a diagnosis of recurrent focal glomerulonephritis and the more acute vascular changes could be accounted for by malignant hypertension. Alternatively and perhaps more likely, the pathology could be explained by repetitive and partially repaired vascular lesions of the type found in TTP (Brain, 1963). More details of this interesting case are given on p. 865 (Case 32).

Shumway and Miller (1957) and MacWhinney, Packer, Miller and Greendyke (1962) described a patient whose history was even more remarkable than that described above. Their patient, a female child, died after a 12-year illness characterized by recurring episodes of hæmolytic anæmia, thrombocytopenia and renal disease. Recurrent infections were followed by exacerbations of her illness and the development of a bizarre (microangiopathic) blood picture. At necropsy, vascular lesions were widespread. MacWhinney and his co-workers (1962) referred also to a second child who died after a 40 days' illness; at necropsy the vascular lesions appeared to be confined to the kidneys.

Two adults whose illness ran relatively long courses were described by Báez-Villaseñor and Ambrosius (1957) and Dreyfus (1958), respectively. The former's patient had had episodes of purpura for 6–8 years prior to a final illness typical of TTP; the latter's patient had repeated neurological symptoms for 2 years before overt TTP supervened. A rather similar case was described by Bornstein, Boss, Casper and Behar (1960), and Jones, Sim and Thomas Smith (1961) reported the history of a patient who had psychiatric symptoms lasting 4 years before death from thrombotic microangiopathy.

Cahalane and Horn (1959) reviewed the course of the illness in 116 patients diagnosed as suffering from TTP and found that fifteen had lived for more than 3 months. They pointed out that the terminal events in long-term cases were usually similar to the occurrences in acute cases.

Further reports of cases of long duration have been published in recent years. For example, a female who died aged 22 had had an illness which probably started when she was 6 years old (*Case Records of Massachusetts General Hospital*, 1963) and other patients whose illness lasted between 1½ and 3 years were described by De Saussure (1964) and Barnhart and co-workers (1964).

Relationship to Systemic Lupus Erythematosus (SLE)

As already mentioned, occasionally, the clinical course and symptoms and/or the pathological findings in life or after death have suggested the presence of both SLE and TTP. Possible cases of this sort were reported by Beigelman (1951) and Gendel, Young and Kraus (1952). Later, Lazlo, Alvarez and Feldman (1955), Siegel, Friedman, Kessler and Schwartz (1957) and Székely (1964) described patients, thought to be suffering from TTP, in whom LE cells were demonstrated.

Recently, Levine and Shearn (1964) have reviewed the supposed occurrence of signs of SLE in cases of TTP. They found that in as many as 34 out of 151 cases of "TTP" (23%) the additional presumptive diagnosis of SLE could be made on the basis of the LE-cell test or the findings at necropsy. In the cases considered to show signs of SLE, 74% had verrucose endocarditis, 24% "onion-skin" changes in the spleen, 21% "wire-loop" glomeruli and 15% positive LE-cell tests; two or more of the above changes were present together in 27%.

Occurrence in Pregnancy

TTP has been reported in pregnancy on a number of occasions (Reisfield, 1959; O'Leary and Marchetti, 1962; *Case Records of Massachusetts General Hospital*, 1963).

Thrombotic Thrombocytopenic Purpura in Childhood

As already described, the hæmolytic-uræmic syndrome is particularly common in childhood, and while it seems to be true that the majority of affected infants and children have not suffered from TTP, some of them probably have. The distinction may be difficult, even on histological grounds, and it is only in cases where vascular lesions can be shown to be widespread that TTP can be positively diagnosed with any certainty. Even so, it has to be emphasized that although a typical case of the hæmolytic-uræmic syndrome in infancy may well have an ætiology which is different from that of a typical case of adult TTP, this has not been proved to be so: the dissimilarities between the syndromes could be the result of qualitative and quantitative differences between the responses of an infant and of an adult to the same ætiological factors, whatever they may be, with consequent differences in the extent and distribution of the vascular lesions.

Selected case reports of children considered to have been suffering from TTP are referred to below.

Singer, Bornstein and Wile (1947) reported the occurrence of purpura and hæmolytic anæmia in an 11-year-old girl, and it was in this paper that they first proposed the title *Thrombotic Thrombocytopenic Purpura.* In retrospect, the patient described by Dacie and his co-workers (1953) as a case of atypical (apparently) congenital hæmolytic anæmia was probably suffering from a chronic relapsing form of TTP. This patient is described as Case 32 (p. 865). Case 3 of Gasser and his colleagues (1955), a child aged 7 months, died after an illness of 30 days. Necropsy was reported to show the lesions of thrombomicroangiopathy (Symmers) as well as bilateral focal renal cortical necrosis. Two of the six children described by Allison (1957) had lesions in other organs as well as in the kidneys and were considered to have suffered from a type of TTP.

Other reports in the literature of children in whom the diagnosis of TTP or Moschcowitz's disease has been made include those of Wile and Sturgeon (1956), Clement and colleagues (1957), Bernheim and colleagues (1957), Robinson, Orchard and Eden (1958), Loeb, Bartman, Dustin and Namèche (1959), Grumbach, Drapeau and Boralevi (1960), Sansone, Buffoni and Rosso (1961), MacWhinney, Packer, Miller and Greendyke (1962) and Swaiman, Schaffhausen and Krivit (1962). [Some, at least, of the infants or young children described in these reports may have in fact been suffering from the hæmolytic-uræmic syndrome of infancy and childhood.]

The report of Clement and his colleagues was of unusual interest as two other siblings had died of an undiagnosed illness, associated with pallor and intestinal hæmorrhage. [All three infants possibly in fact suffered from the hæmolytic-uræmic syndrome of infancy.] Robinson, Orchard and Eden's report dealt with a young child whose illness was remarkable for a prolonged (2-year) course and continued pyrexia. Drug sensitivity appeared to be a possible ætiological factor.

MacWhinney and his co-workers (1962), who reviewed the occurrence of TTP in childhood, found reports of 19 cases where the diagnosis of TTP could be sustained on histological grounds; six of the children were less than 1 year of age. Less than half of them had neurological symptoms and thrombocytopenia was sometimes absent or minimal. The disease as it affects children thus appears to differ from that in adults in the relative infrequency of neurological signs or symptoms.

The Blood Picture and other Laboratory Findings in Thrombotic Thrombocytopenic Purpura

Erythrocytes. Anæmia is probably invariably present at some stage in the course of every patient's illness. Often it is very severe and may dominate the clinical picture. The blood picture is similar to that seen in the hæmolytic-uræmic syndrome of infancy.

The number of abnormally shaped and fragmented cells present varies from case to case in direct proportion to the severity of the anæmia; often the changes are marked and the general appearance of the film may be most bizarre (Figs. 215 and 216) (see also p. 882, under *Microangiopathic Hæmolytic Anæmia*). Polychromasia in proportion to the reticulocyte count will be present and as a rule small numbers of erythroblasts. Auto-agglutination is not a feature of the films, although rouleaux may be present and sometimes an abnormal degree of crenation. Punctate basophilia also is not frequently seen.

The reticulocyte count is usually considerably raised and counts of 30% or more are not infrequent. In Romanowsky-stained films the reticulocytes appear as diffusely basophilic round macrocytes which contrast markedly with the distorted and contracted orthochromic adult erythrocytes.

Leucocytes. A neutrophil leucocytosis is commonly found and metamyelocytes and a few myelocytes may be present also. Occasionally, the total leucocyte count may reach as high as 50,000 per cu. mm. giving rise to a "leukæmoid" blood picture. On the other hand, in at least two instances of probable TTP, leucopenia has been recorded (Brittingham and Chaplin, 1957; Bukowsky and Koblenzer, 1962), and in one case a high eosinophilia (Fisher and Creed, 1955).

Platelets. Thrombocytopenia is the typical finding in TTP, but the counts are variable and fluctuate quite widely in individual patients. In chronic cases the count may be normal in remission, only to fall sharply in exacerbations of the disease. In acutely ill patients platelets may be virtually absent; however, counts in the range 10,000–30,000 per cu. mm. are more usual. Occasionally in adults the platelet count is hardly lowered and, as already remarked on, an absence of marked thrombocytopenia may be more frequent in childhood (MacWhinney *et al.*, 1962).

Osmotic and Mechanical Fragility. Moderate to marked increases in osmotic fragility have been repeatedly reported. The changes—often a tail of fragile cells in the osmotic fragility curve—reflect the presence of the contracted and distorted and spherocytic erythrocytes seen in the blood films. Singer (1954), in his review, stressed how the results of both osmotic and mechanical fragility tests fluctuated in individual patients. Shumway and Miller (1957), too, remarked on how clinical exacerbations in hæmolysis were accompanied in their patient by increases in osmotic and mechanical fragility and also by a slight increase in the rate of autohæmolysis.

Other Laboratory Findings

Serum Bilirubin. The levels usually range between 0·8 and 3·0 mg. per 100 ml.

Plasma Hæmoglobin and Hæmoglobinuria. The plasma-hæmoglobin levels have seldom been reported on, but considerably raised levels are to be expected, as there seems little doubt but that hæmolysis occurs, to some extent at least, intravascularly. Overt hæmoglobinuria, however, is not commonly found; it occurs perhaps more commonly in children than in adults, *Plasma Proteins.* Low fibrinogen levels and hypoprothrombinæmia were reported by Bukowksi and Koblenzer (1962) in a chronic case and deficiencies in plasma globulins were described by Frick and Hitzig (1959) in three out of five patients: one patient had congenital agammaglobulinæmia and another absence of β_2M globulin.

Barnhart, McCutcheon, Riddle and Ohorodnik (1964) in an interesting paper reported on studies on protein synthesis in a patient who died of fulminating TTP. There was evidence for a hypercoagulable state: the thrombin time was shortened and the fibrinogen concentration raised to 400 mg. per 100 ml. The γ-globulin concentration was raised (1·4 g. per 100 ml.). It was concluded, by the use of fluorescent antibody techniques, that fibrinogen, prothrombin and albumin were being synthesized at an enhanced rate in the liver.

Serology

Evidence for the presence of abnormal antibodies acting against erythrocytes and/or platelets has been repeatedly sought. The results have been almost uniformly negative. Nevertheless, positive antiglobulin tests have been reported in a few instances (*e.g.*, by Lennox and Dacie (1951); Báez-Villaseñor and Ambrosius (1957); Ritz, Groisser and Banowitch (1956); Chaplin (1959)); the reactions have, however, been weak and, where tested, of the non-γ type (Lennox and Dacie, 1951; Chaplin, 1959). The rarity of positive antiglobulin tests needs stressing—Cahalane and Horn (1959) found only six positive tests in their review of 116 patients—in view of suggestions that the hæmolytic anæmia of TTP might have an auto-immune origin (see p. 869).

Erythrocyte Survival Studies

Only a few studies appear to have been carried out. Singer, Motulsky and Shanberge (1950) concluded on the basis of patients' transfusion requirements that normal erythrocytes were destroyed at a rapid rate. This view has been confirmed by more specific studies. Dacie and his co-workers (1953) reported, in their chronic case, that the elimination of normal cells was complete in 50 days, while Adelson, Heitzman and Fennessey (1954) observed in an acute case that transfused normal erythrocytes, as well as transfused platelets, were rapidly eliminated.

Shumway and Miller (1957) made some interesting observations: when their patient was acutely ill less than 1% of transfused normal cells were circulating after 16 days; but in a temporary remission (after splenectomy) elimination was not complete until 50 days had elapsed.

Brittingham and Chaplin (1957) found that the ^{51}Cr T$\frac{1}{2}$ of their patient's erythrocytes was 6·5 days. Later, Chaplin (1959), referring (presumably) to the same patient, reported that the plasma hæmoglobin was 100 mg. per 100 ml. at the time of the experiment and that, although no evidence of excess splenic accumulation of radioactivity could be detected by external counting, the spleen (after splenectomy) contained four times the radioactivity per gram compared with the liver.

Swaiman, Schaffhausen and Krivit (1962) obtained some interesting results by a cross-transfusion experiment. The ^{51}Cr T$\frac{1}{2}$ of normal erythrocytes transfused to the patient was very short, *i.e.*, 3 days, but that of the patient's cells transfused to a normal subject was much longer: the ^{51}Cr T$\frac{1}{2}$ was 15–17 days, clearly demonstrating the extracorpuscular nature of the hæmolytic mechanism.

Pathology of the Vascular Lesions of Thrombotic Thrombocytopenic Purpura

A detailed account of the vascular lesions found in TTP would be out of place in this book. However, a brief description will be given. As described and illustrated in the original papers of Moschcowitz (1924, 1925) and Baehr, Klemperer and Schifrin (1936), eosinophilic "hyaline thrombi" are present in many small arterioles and arteriolar capillaries, particularly in those of the kidneys, brain, myocardium and adrenals, and of the liver, spleen and pancreas; in fact they have been observed in most tissues of the body including the skin and bone marrow (Ruffolo, Pease and Cooper, 1962) and lymph nodes (Morey, White and Daily, 1956). The thrombi usually occupy most of the lumen of the vessels and may be hyaline in appearance or granular; not infrequently, the lumina of the vessels are completely obstructed. The intimal lesions may be associated with local endothelial proliferation, but there is usually little or no perivascular infiltration although there may be nearby hæmorrhage. However, the elastic lamina of affected arterioles may be destroyed and aneurisms have been demonstrated at arteriolar-capillary junctions (Orbison, 1952). Hyaline, or sometimes fibrinoid, material may be found beneath the endothelium of vessels apparently free from thrombi (Symmers, 1952).

The nature of the hyaline or granular thrombi has been a subject of dispute. Moschcowitz (1924, 1925) originally conceived that the thrombi were composed largely of agglutinated erythrocytes; subsequently, masses of platelets were thought to be the main component (Baehr, Klemperer and Schifrin, 1936; Altschule, 1942; Trobaugh, Markowitz and Davidson, 1946). More recently, most authors have concluded that they are made largely of fibrin (Stuart and MacGregor-Robertson, 1956; Craig and Gitlin, 1957) and that in all probability the fibrin is deposited on the surface of damaged endothelium or vessel wall (Altschule, 1942; Singer, 1954; Moore and Schoenberg, 1960).

Case Report: Fatal Thrombotic Thrombocytopenic Purpura

Case 31. The patient, R. H., a young woman aged 24, was admitted to Hammersmith Hospital in semi-coma on the 6th July 1959. She had felt unwell 2 weeks before admission and from thereon she had suffered from several transient attacks of hemianopia and of tingling of the right side of the face and hand. She had also had several attacks of transient dysphasia. Ten days before admission she had been noticed to be slightly jaundiced. Three days before admission she had become drowsy and disorientated and had bled from her gums; finally, she had vomited twice and had lost the power of speech.

Physical Examination. On admission she was found to be very pale and slightly jaundiced. The temperature was 101·4° F. She was in semi-coma but was restless and her eyes moved from side to side and she uttered low moans; she responded, however, to painful stimuli by movements of all four limbs; her neck was stiff.

Purpura was present on the face, arms and trunk. Neither the spleen nor the liver was palpable. The cardiovascular and respiratory systems were normal; the B.P. was 110/70.

Laboratory Findings. On admission, the hæmoglobin was 6·8 g. per 100 ml., PCV 18·5%, and there were 7·2% reticulocytes. The leucocyte count was 7,000 per cu. mm., with 69% neutrophils, and the platelet count 9,000 per cu. mm. Stained blood films showed the changes of microangiopathic hæmolytic anæmia: there was anisocytosis, spherocytosis and polychromasia, and distorted and contracted cells were present in moderate numbers (Fig. 215).

Bone-marrow puncture showed an active normoblastic marrow and an excess of megakaryocytes, but there was little evidence of platelet formation.

The direct antiglobulin test was negative and erythrocyte osmotic fragility was normal, lysis commencing in 0·5% NaCl. Serum proteins were 7·1 g. per 100 ml., albumin 4·3 g. and globulins 2·8 g. Serum bilirubin was 2·7 mg. per 100 ml. Tests for antinuclear factor were negative. The W.R. was negative.

Methæmalbumin was demonstrated in the serum and haptoglobins were absent. The blood urea was 46 mg. per 100 ml.

The urine contained protein and excess urobilinogen, and leucocytes and erythrocytes were seen in the deposit.

Progress and Treatment. A diagnosis of thrombotic thrombocytopenic purpura was made on the basis of the clinical and laboratory findings.

She was transfused with 1 l. of blood but this had little effect on the blood picture and by the 10th July the hæmoglobin had fallen back to 6·9 g. per 100 ml., and the reticulocyte count had risen to 24%. The platelet count remained very low throughout (7,000–17,000 per cu. mm.). An erythrocyte auto-survival study using ^{51}Cr showed the mean cell life-span to be greatly shortened (approximately 5 days).

She was treated with intravenous hydrocortisone (300 mg. daily) but she slowly became more deeply comatose and died 5 days after admission. The blood urea was 63 mg. per 100 ml. on the day before death.

Necropsy Findings. The immediate cause of death was cerebral and pulmonary œdema. Histological examination showed that the typical vascular lesions of TTP were widely distributed.

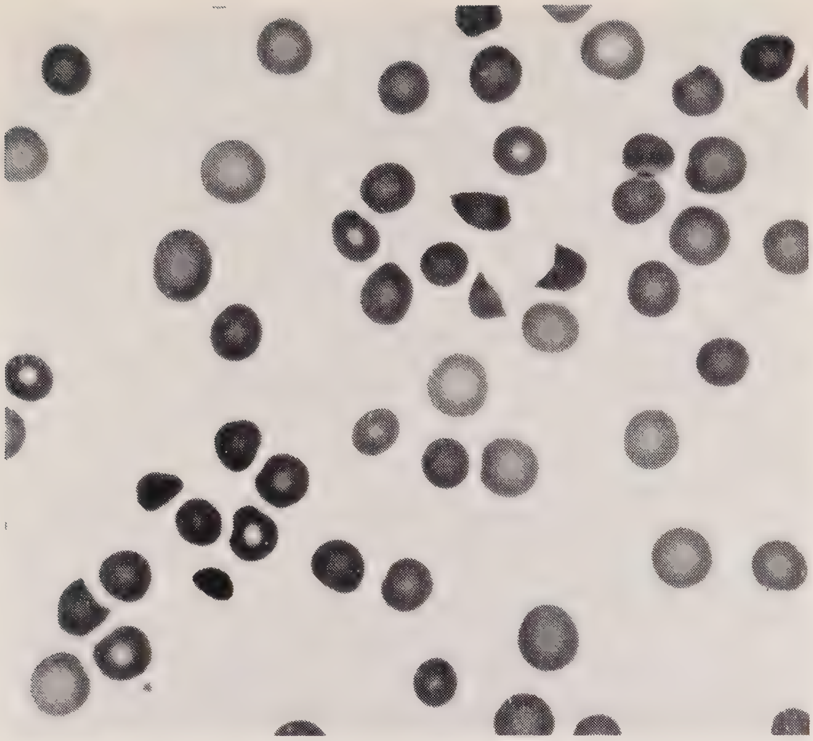

Fig. 215. Photomicrograph of a blood film of a patient who died of thrombotic thrombocytopenic purpura (Case 31). There is considerable anisocytosis and a tendency to spherocytosis. Densely-staining contracted cells of irregular shape are present in moderate numbers. × 700.

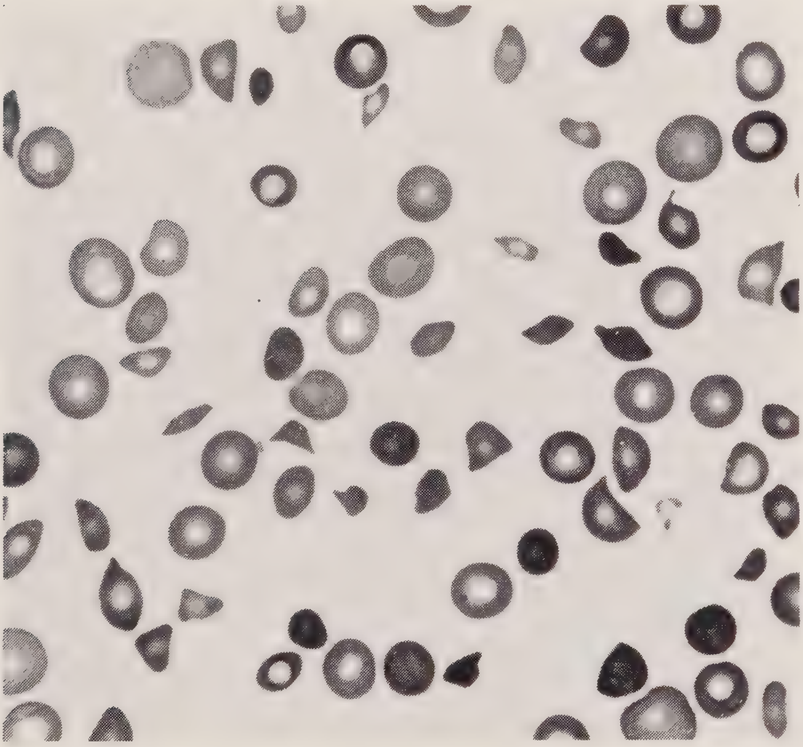

Fig. 216. Photomicrograph of a blood film of a patient who died probably of a chronic form of thrombotic thrombocytopenic purpura (Case 32). There is marked anisocytosis and many contracted cells of irregular outline, including "burr" cells and triangular forms, are present. × 700.

Hyaline thrombi were seen in arterioles in the brain, adrenals, pancreas, heart, posterior pituitary, œsophagus, stomach and kidneys where they were numerous in interlobular arteries and present also in some of the afferent arterioles.

Summary. A case of acute thrombotic thrombocytopenic purpura. Death in coma occurred within 3 weeks of the start of the illness. Renal failure was not a marked feature.

Case Report: (?) Chronic Thrombotic Thrombocytopenic Purpura in a Child: Death from Malignant Hypertension

Case 32. The patient, V. G., died in Hammersmith Hospital on the 28th December 1950 when almost 7 years of age, after an illness which had lasted for at least 3 years and almost certainly longer.[1] She was the third child of parents who were second cousins. Both their first and second children had died of hæmorrhage, the first when aged 2 years following the extraction of a foreign body from the nose and the second child from intestinal hæmorrhage on the 4th day of life. A fourth child had developed normally.

The patient V. G. had been jaundiced at birth and had had purpura and was noted to be pale when aged 7 months. A similar episode had occurred following measles at the age of 2 years. When 3½ years she was reported to have had tonsillitis and when 4½ years a sore throat was accompanied by pallor and hæmaturia and there were widespread bruises and petechiæ. She was found to be anæmic and transfused, and erythrocytes, granular casts and protein were found in the urine. She was discharged from Hospital in March 1948 but further attacks of purpura occurred in May and August following upper respiratory tract infections.

Thrombocytopenia had been found and this led to splenectomy in February 1949. The platelet count temporarily rose to supranormal levels, but thrombocytopenia and anæmia soon recurred and this led to a further admission to Hospital. On the 6th December 1949 she was admitted to Hammersmith Hospital for further study.

Physical Examination. On admisson she was found to be pale and to have fading bruises on the legs, and there was clinical evidence of cardiac enlargement. The liver was palpable 3 cm. below the right costal margin. Lymph nodes were not significantly enlarged. The B.P. was 135/75 mm. Hg.

Laboratory Findings. The erythrocyte count on admission was 2,400,000 per cu. mm., hæmoglobin 6·6 g. per 100 ml., PCV 19% and reticulocytes 15%. The leucocyte count was 9,000 per cu. mm. and the platelet count 240,000 per cu. mm. Blood films showed a remarkable picture (Fig. 216), many of the erythrocytes being strikingly contracted and distorted, and angular and triangular forms were conspicuous. Polychromasia was also a conspicuous feature, the young cells staining basophilically being macrocytes and generally perfectly round in contour.

The erythrocyte osmotic fragility was raised, there being 15% lysis in 0·55% NaCl and a trace of lysis in 0·70% NaCl. The direct antiglobulin test was negative.

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[1] An account of this patient was given by Dacie and colleagues (1953, Case 12), and a fuller description was included in Brain's (1963) Thesis.

The serum bilirubin was 0·7 mg. per 100 ml. and serum proteins 7·0 g. per 100 ml., albumin 4·1 g. and globulins 2·9 g. The blood urea was 51 mg. per 100 ml. The urine contained 240 mg. protein per 100 ml., and occasional erythrocytes and leucocytes and hyaline and granular casts were seen in the deposit.

She was given a blood transfusion and the survival of the transfused blood was followed by the Ashby method. The normal erythrocytes survived subnormally, all the cells being eliminated by the 50th post-transfusion day.

Further Progress (Fig. 217). The child was discharged from Hospital on the 20th December 1949 with a hæmoglobin of 15·2 g. per 100 ml. and a platelet count of 270,000 per cu. mm. However, she soon developed an upper respiratory tract infection and was readmitted into Hospital on the 12th January 1950, again anæmic and thrombocytopenic: the hæmoglobin was 7·4 g. per 100 ml. and the reticulocyte count 21%; the platelet count was 70,000 per cu. mm. She was transfused and discharged only to be readmitted 3 weeks later in much the same condition. The urea clearance was 18·5% of normal although the blood urea was only 56 mg. per 100 ml.

Two further admissions were required in May and June 1950. During the latter admission a group-A *Str. pyogenes* was cultured from the throat and the anti-streptolysin titre markedly rose. She was treated with sulphonamides and was kept on a prophylatic dosage subsequently.

Each of the above episodes was associated with an increase in hæmolysis, and on recovery from the 2nd episode the reticulocyte count rose to as high as 46%. The strikingly abnormal erythrocyte morphology persisted throughout, but was more marked during the periods of more active hæmolysis.

She was readmitted into Hospital in August 1950 for reassessment. A new finding was a rise in blood pressure to 160/100 mm. Hg and a small rise in the level of the blood urea to 63 mg. per 100 ml. In October 1950 the blood pressure had risen further to 190/130 mm. Hg. She was admitted to Hospital for the last time in December 1950 because of persistent vomiting and hæmaturia. She had by then developed severe hypertensive retinopathy. The hæmoglobin was 8·9 g. per 100 ml., with 30% reticulocytes and there were 13,000 platelets per cu. mm. The blood urea was 162 mg. per 100 ml. Three days after admission she lost consciousness temporarily and after recovery was found to have developed a left hemiplegia. The blood pressure was then 240/140 mm. Hg. Her urinary output remained at a reasonable volume but the blood urea nevertheless rose and had reached 205 mg. per 100 ml. at the time of her death on the 28th December 1950.

Necropsy Findings. The main macroscopic findings were an area of softening in the right parietal region of the brain, left ventricular hypertrophy, a finely granular surface to the kidneys and hyperplastic red bone marrow. Histological examination showed the presence of fibrin thrombi in arterioles and capillaries in the brain, myocardium, kidneys and intestinal tract (œsophagus and duodenum). Major changes were present in the renal vessels: the majority of the afferent arterioles showed fibrinoid necrosis of their walls, with destruction of elastic tissue, and a few were aneurysmal. Fresh thrombi were present in many arterioles, and in some the thrombi had been organized. The glomeruli were mostly hypercellular and in some polymorphonuclear leucocytes were conspicuous; there were occasional focal capsular ad-

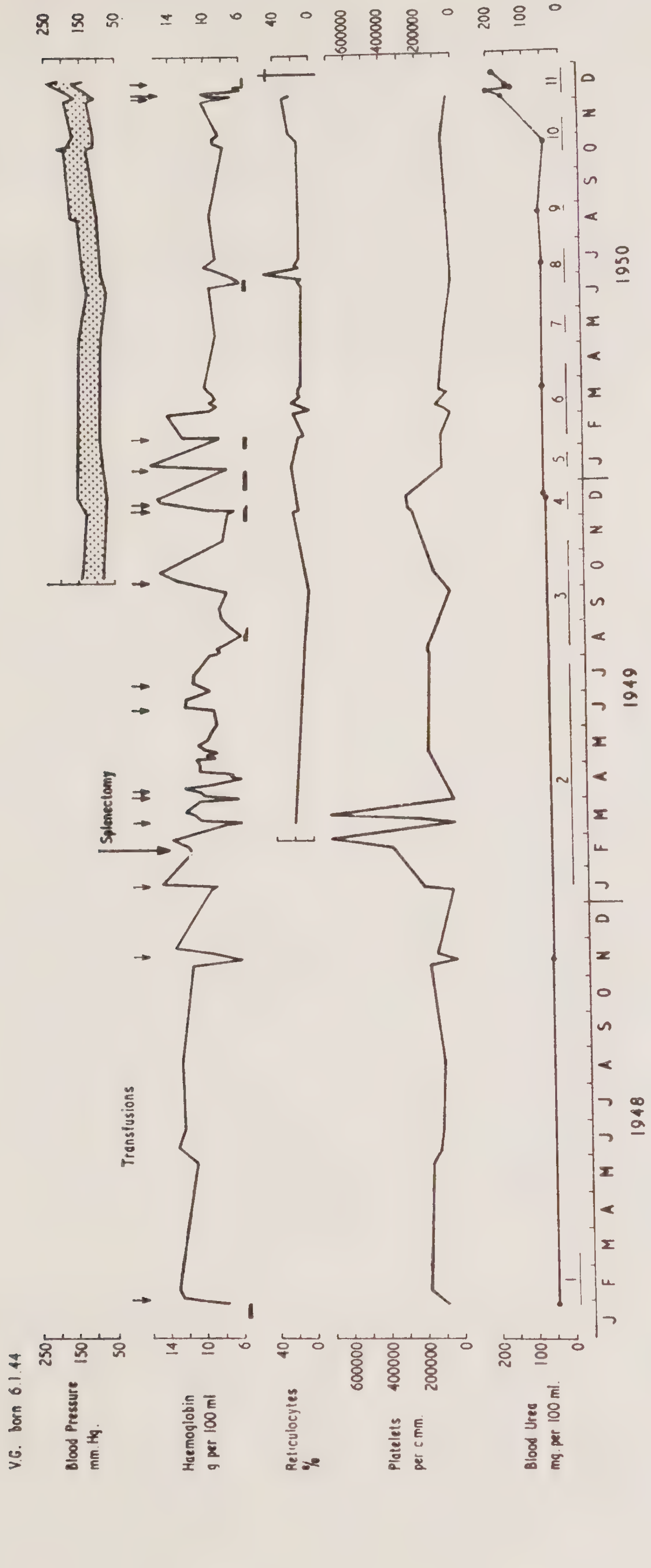

FIG. 217. Diagram illustrating the clinical course of, and laboratory findings in, a patient who died probably of a chronic form of thrombotic thrombocytopenic purpura (Case 32). (The figures 1 to 11 refer to hospital admissions.)

hesions but no crescents or periglomerular fibrosis. The capillary loops were matted together in many of the large cellular glomeruli and their basement membranes were thickened. Scattered glomeruli had become totally replaced by collagenous tissue. Hæmosiderin was demonstrated in tubular cells in the cortex.

The histological findings were considered to be compatible with chronic thrombotic thrombocytopenic purpura which had led to recurrent focal vascular lesions in the kidney, on top of which the changes of malignant hypertension had been superimposed.

Summary. A case of chronic thrombotic thrombocytopenic purpura, with a well-marked microangiopathic hæmolytic anæmia blood picture. No response to splenectomy. Exacerbations in hæmolysis and thrombocytopenic purpura were associated with upper respiratory tract infections. Death resulted from the effects of an apparently superadded malignant hypertension and renal failure.

ÆTIOLOGY AND PATHOGENESIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA

The Possible Role of Allergy. A detailed consideration of the development of views on the ætiology and pathogenesis of TTP is beyond the scope of this book. Perhaps it is sufficient to say that most writers, as well as the present author, now conceive the disorder to be allergic in origin, with the vascular endothelium of small blood vessels being primarily damaged (Altschule, 1942; Singer, 1954; Antes, 1958, etc.), and a possible analogy with the Shwartzman reaction (Shwartzman, Klemperer and Gerber, 1936) has been repeatedly commented on (Shwartzman, Klemperer and Gerber, 1936; Altschule, 1942; Trobaugh, Markowitz and Davidson, 1946; Brunson and Davis, 1955; Taub, Rodriguez-Erdmann and Dameshek, 1964).

It should be added that Hjort and Rapaport (1965) concluded, on the other hand, that although certain human disorders probably were analogous to the Shwartzman reaction, TTP was unlikely to be one such condition; they pointed out that in all probability the vascular lesions in TTP came first and that the thrombi were secondary events.

A close relationship to the "collagen" diseases has also often been discussed (see Singer, Motulsky and Shanberge, 1950; Beigelman, 1951; Symmers, 1952; Levine and Shearn, 1964). In a number of cases, too, a drug hypersensitivity seems to have been a probable ætiological factor (Engel, Scheinker and Humphrey, 1947; Monroe and Strauss, 1953; Symmers, 1956b, 1962; Robinson, Orchard and Eden, 1958).

Pathogenesis of the Hæmolytic Anæmia and Thrombocytopenia

Even if an allergic mechanism leading to vascular damage is proved to be the correct explanation for the pathogenesis of TTP, the problem of the accompanying hæmolytic anæmia and thrombocytopenia remains to be answered. The hypothesis that the same agent or mechanism which damaged vascular endothelium might also damage erythrocytes and platelets (Singer, Motulsky and Shanberge, 1950) is still, as Singer and his colleagues put it, an "assumption not based on any established facts." Two facts weigh strongly against the hypothesis that antibody is developed against the patient's erythrocytes: one is that the direct antiglobulin test is almost always clearly negative, and the second is that the morphology of the erythrocytes is unlike that found in genuine auto-immune hæmolytic anæmia where, although spherocytosis is often seen, the bizarre cell distortion and fragmentation which is a typical feature of TTP is absent.

The evidence, too, is against the hypothesis that the thrombocytopenia is based on an immunological mechanism, even if the negative results of tests for platelet antibodies are discounted. On at least two occasions the transfusion of substantial volumes of plasma from a patient with TTP to a normal recipient has failed to cause a fall in the recipient's platelet count (Adelson, Heitzman and Fennessey, 1954; Brittingham and Chaplin, 1957).

Faced with the lack of evidence in favour of an auto-immune mechanism for the hæmolysis, or indeed in favour of any reasonable existing explanation, Brain, Dacie and Hourihane (1962) suggested that the damage to the erythrocytes might occur locally within the diseased blood vessels, as the result of physical or chemical insult sustained while the cells were in close contact with an abnormal environment. This hypothesis is considered in more detail on p. 887. In this connection it is fair to recall that Monroe and Strauss (1953), in describing the necropsy findings in two cases of TTP, noticed appreciable numbers of erythrocyte fragments in tissue sections and concluded that they were actually seeing erythrocytes in the course of being destroyed: they reported that "schizocytes" were most numerous at the sites of vascular lesions.

Role of Azotæmia. There is no evidence that uræmia or azotæmia *per se* is responsible for the hæmolytic anæmia in TTP or indeed for the morphological changes in the erythrocytes. Shumway and Miller (1957), in their detailed study of a child suffering from a chronic relapsing form of TTP, were unable to correlate the striking erythrocyte abnormalities present with nitrogen retention, and this was clearly shown, too, by Brain, Dacie and Hourihane (1962) in their survey of a large number of cases of microangiopathic hæmolytic anæmia (Fig. 218).

The cause of the thrombocytopenia of TTP has not yet been clearly settled. Bone-marrow studies have shown an excess rather than a paucity of megakaryocytes (Singer, Motulsky and Shanberge, 1950), and a peripheral destructive mechanism seems probable. Most authors have felt that platelet production from megakaryocytes is not impaired; but there have been, nevertheless, some reports to the contrary (*e.g.*, Burke and Hartmann, 1959). At a time when the intimal thrombi were considered to be composed of platelets, their removal at the sites of thrombus development seemed to provide a reasonable explanation for the thrombo-

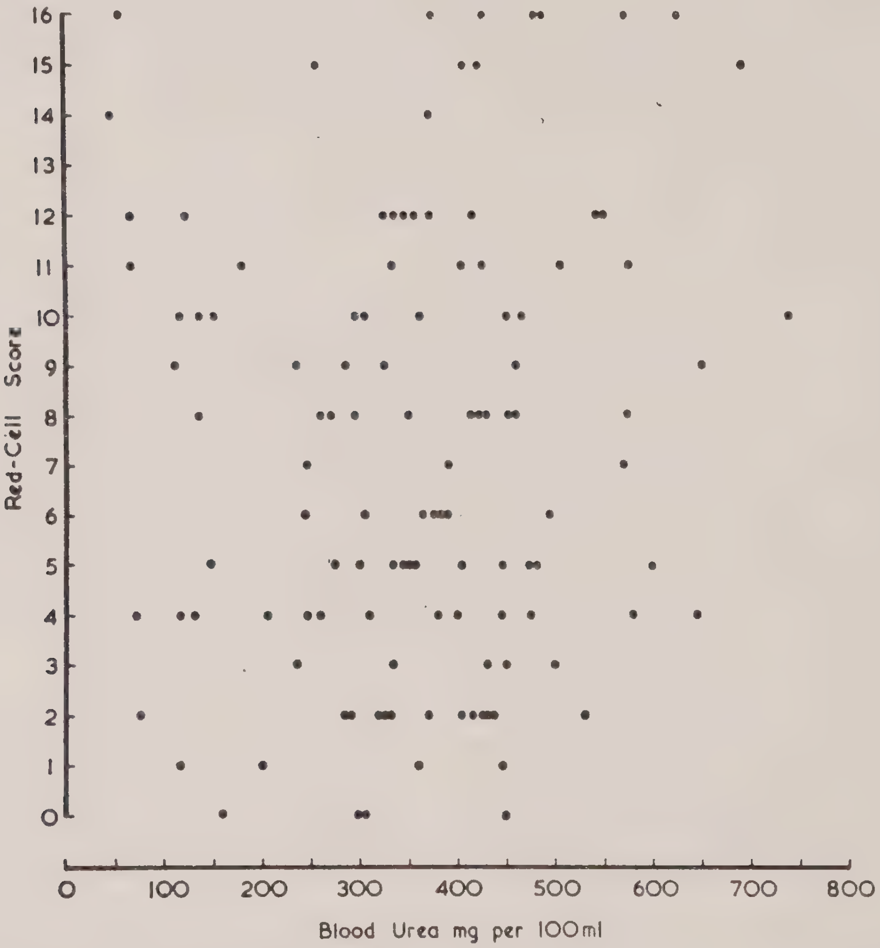

Fig. 218 Relationship between the severity of the erythrocyte abnormalities and blood urea in 115 patients, all except two of whom had been admitted to hospital because of acute or chronic renal failure. There is no correlation between the two parameters ($r=0\cdot007; 0\cdot5>P>0\cdot4$).

The severity of erythrocyte change is scored from 1 to 16 (From Brain, Dacie and Hourihane, 1962).

cytopenia. The more recent view (see p. 863) that the thrombi consist largely of fibrin certainly makes this hypothesis less easy to sustain. It is possible, however, that the platelets are removed from the circulation more widely as the consequence of their being utilized in a process of continuous intravascular clotting (defibrination) which is initiated at the site of vascular damage. Evidence in favour of this hypothesis has been furnished by Barnhart, McCutcheon, Riddle and Ohorodnik (1964).

Another mechanism which might provide a part explanation for the thrombocytopenia is the liberation of clotting-promoting substances from the damaged and lysed erythrocytes (Swaiman, Schaffhausen and Krivit, 1962) or from reticulocytes (Bradlow, 1961). Research to test the validity of these hypotheses is urgently required.

It is interesting to record that Renaud (1962) reported that male rats kept for several months on a high fat diet containing cholic acid developed "thrombotic thrombocytopenia." Histological examination of their organs at necropsy shows changes reminiscent of TTP: namely, widespread thrombi in small vessels, aneurisms and sometimes rupture of the vessel wall. The platelets fell to about one-quarter of the normal value and hæmolytic anæmia with spherocytosis (but little poikilocytosis) developed before death. It is tempting to believe that here, too, the thrombocytopenia and hæmolytic anæmia occurred secondarily to the microthromboses which under the experimental conditions were of dietary origin.

TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA

It is extremely difficult to assess the value of attempts to treat patients with TTP, but as it seems clear that death is *not* inevitable in every case the possibilities of benefit by energetic treatment cannot be neglected. The logical course would be to combat the underlying basis of the disease if possible, while controlling palliatively its main consequences, *i.e.*, the anæmia, thrombocytopenia and uræmia. In view of the probable allergic ætiology of the vascular damage, intensive corticosteroid therapy would at the present time seem to be a logical form of treatment.

Steroid Therapy. Unfortunately, the results of treatment with ACTH or adrenocorticosteroids have usually been disappointing, even when the drugs have been used in large doses. However, some successes have been claimed. Two such responses were reported by Burke and Hartmann (1959), using large but orthodox doses of the drugs (the maximum dose of prednisone was 100 mg.

daily). The clinical charts illustrating the responses of these two patients to treatment are quite impressive. While not claiming that they had proved that the patients' remissions had in fact been induced by the treatment they had received, the authors concluded with a plea for further trial of the effects of large doses of steroids (*e.g.*, 100 mg. of prednisone daily) given early in the disease, a recommendation with which the present writer agrees. Two further cases treated successfully by high-dose steroid therapy were reported by Hill and Loeb (1960). Improvement in one patient appeared to be initiated by 150 mg. of ACTH given intravenously, 60 mg. of ACTH given intramuscularly and 300 mg. of prednisolone given intravenously, all simultaneously during a 24-hour period.

De Saussure (1964) described a patient who apparently responded to 240 mg. of prednisone daily. (It has to be added that this patient died 15 months later from an acute relapse and that on this occasion steroid therapy was ineffectual.) Even larger doses have been recommended by Taub, Rodriguez-Erdmann and Dameshek (1964), who stated that massive steroid therapy—up to 1,000 mg. of prednisone daily—might be of value; in four cases this regime, followed by splenectomy, led to complete (or incomplete) recovery. The steroids were thought to prevent intravascular coagulation by their inhibiting effect on antigen-antibody reactions. The author feels that few physicians would wish to follow this drastic course—and that the same end-result might be obtained by the bold use of heparin (see below).

Splenectomy. This has been carried out on a number of patients, and it is quite clear that in most of them the operation has failed to influence the course of their disease. But again however, as with steroid therapy, some successes have been reported. Thus, the second patient of Meacham and his co-workers (1951) appeared to develop a 2-year remission after splenectomy, and similar long-lasting remissions have been reported by Siegel and co-workers (1957), Shapiro, Doktor and Churg (1957), Rodriguez, Babb, Santiago and Costas-Durieux (1957) and Moorhead (1966). The two patients of Hill and Loeb (1960), who appeared to respond to steroids, subsequently went into complete remission after splenectomy.

The effect of splenectomy was reviewed by Cahalane and Horn (1959), who found records of 18 such operations, eight being carried out on patients whose disease was running a protracted course. Remissions followed in five out of the 18 patients. As already mentioned above, Taub, Rodriguez-Erdmann and Dameshek (1964) have recommended "quick splenectomy" following massive steroid therapy. Recently, the case histories of three more patients successfully treated in this way were described by Distenfeld and Oppenheim (1966).

It is difficult to believe that splenectomy could influence substantially the course of the disease in a patient whose vascular lesions were widespread, but if such lesions were predominantly located within the spleen, then a favourable response might well follow. Perhaps splenectomy should be considered more often than it has been in the past, particularly in the less acutely ill patient whose spleen is palpably enlarged.

Heparin Therapy. Experimentally, the effects of the Shwartzman reaction can be neutralized by the administration of heparin (Good and Thomas, 1955; Brain and Hourihane, 1967).

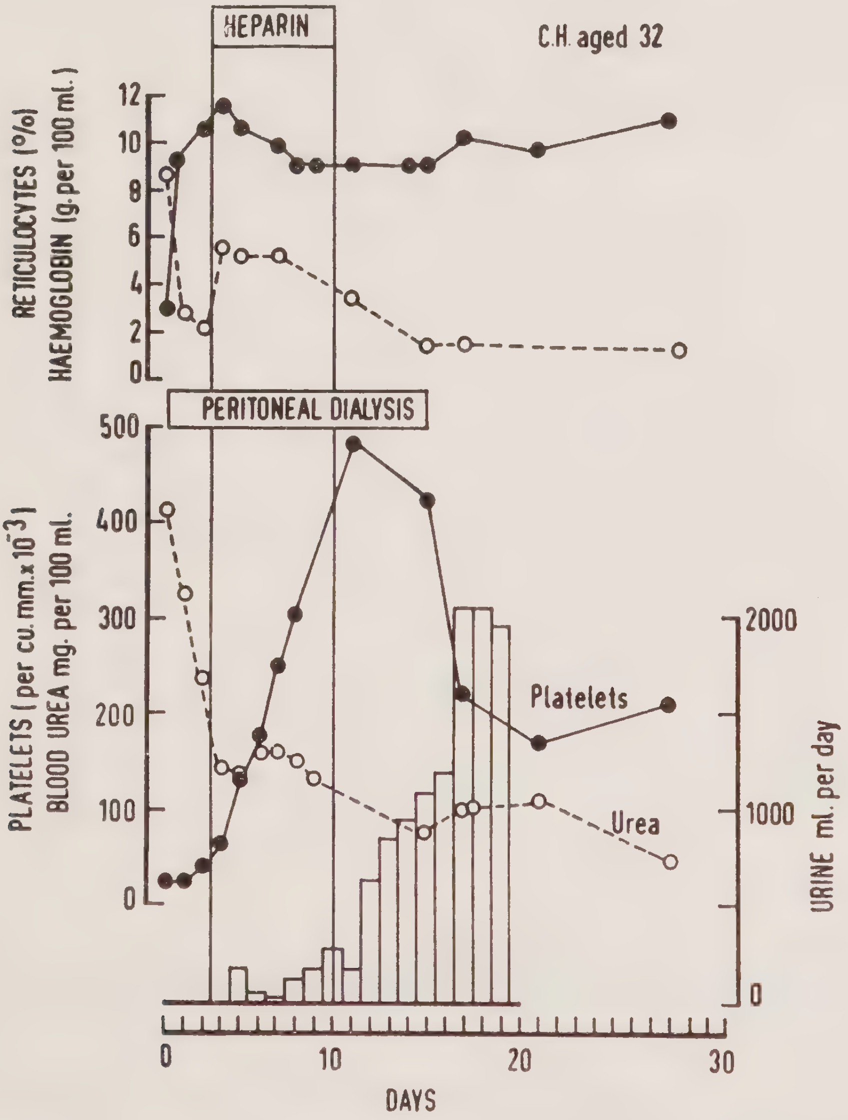

FIG. 219. Response to heparin therapy (5,000 i.u. 8-hourly) in a patient (C. H.) suffering from acute renal failure and microangiopathic hæmolytic anæmia of unknown origin.

Top half of chart: continuous line = hæmoglobin level; interrupted line = reticulocyte count.

The use of heparin as treatment for human TTP was first suggested apparently by Singer (Singer, Bornstein and Wile, 1947; Singer, Motulsky and Shanberge, 1950), but it has seldom been employed. Adelson and Stefanini (1952) and Adelson, Heitzman and Fennessey (1954) used it, but for only a short period, without success in two patients.

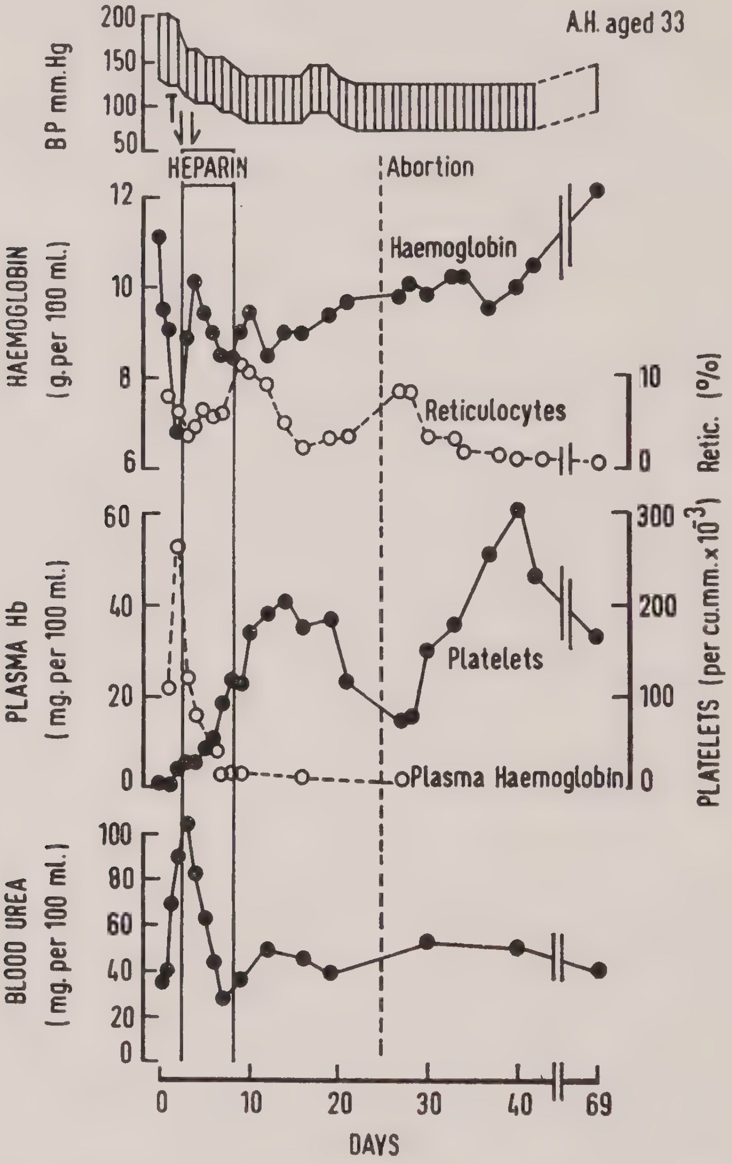

FIG. 220. Response to heparin therapy (20,000 i.u. 6-hourly) in a patient (A. H.) suffering from pre-eclamptic toxæmia, microangiopathic hæmolytic anæmia and severe thrombocytopenia.

Bernstock and Hirson (1960) were the first to persevere with heparin and to report an apparent successful response: on two successive occasions dramatic remissions occurred whilst their patient was being treated with daily intramuscular injections of heparin for 4 weeks and 12 days, respectively. (Bernstock (quoted by Allanby, Huntsman and Sacker, 1966), however, later stated that his patient relapsed again, while pregnant, and died despite further treatment with heparin.)

More recently, Allanby, Huntsman and Sacker (1966) have described an apparent response to heparin (combined with magnesium therapy) in a male aged 35. This patient was reported as being well 3½ years later. Rodriguez-Erdmann, Rubin and Dameshek (1966), too, have reported another apparent response to heparin in a patient also treated by prednisone in high doses and splenectomy.

Brain (1966) has treated five patients with heparin. Three were children suffering from the hæmolytic-uræmic syndrome and two were adults, one suffering from acute renal failure of unknown origin and the other from pre-eclampsia. One of the children recovered and it appeared that the heparin may have played a part in his recovery (Case 29, p. 850); the other two children, who received small doses of heparin, died. Both adults recovered and in each case the heparin therapy appeared to be a major factor in bringing this about (Figs. 219 and 220).

Heparin therapy, in the writer's opinion, deserves further trial in the treatment of TTP, particularly also in view of the reported possible responses of infants and children suffering from the hæmolytic-uræmic syndrome (see p. 845); and the reluctance to give the drug to patients already suffering from a hæmorrhagic diathesis should not be allowed to weigh too heavily against its use. If fibrin deposition on vascular endothelium is the basis for the vascular blockage and consequent renal failure, and indirectly for the hæmolytic anæmia and thrombocytopenia, then anticoagulant therapy, by helping to prevent fibrin formation, might well arrest the progress of the disease.

Blood Transfusion. Fresh blood and/or platelet concentrates should be used to combat anæmia and to replace platelets, as required. Exchange transfusion in one case appeared to initiate recovery after steroid therapy had apparently failed (Rubinstein *et al.*, 1959).

HÆMOLYTIC-URÆMIC SYNDROME IN ADULTS: II. IN PATIENTS NOT SUFFERING FROM THROMBOTIC THROMBOCYTOPENIC PURPURA

Malignant Hypertension. A small number of cases of severe anæmia associated with a raised reticulocyte count, other evidence for excess hæmolysis and a microangiopathic hæmolytic anæmia picture have been reported in patients suffering from terminal and acutely progressive malignant hypertension. Dacie (1954) described two such patients: in the first patient (Case 25 (1954, p. 368)) the hypertension was a sequel to pre-eclampsia; the second (Case 27 (1954, p. 369)) was a male aged 35 years (Figs. 221 and 223). In this last patient there was an intense fibrinoid necrosis of the walls of the renal interlobular arteries and of afferent glomerular arterioles. Many of the glomeruli showed endothelial-cell proliferation and some a variable degree of hyalinization of the capillary tufts. Brain (1963) described a further patient, a woman aged 49, in whom the blood picture and renal histology findings were similar; and another patient from the Postgraduate Medical School has been reported more recently (*Clinico-Pathological Conference,* 1964). More recently, additional cases have been described by Sussman and French (1965) and Capella, Wesson and Erslev (1966).

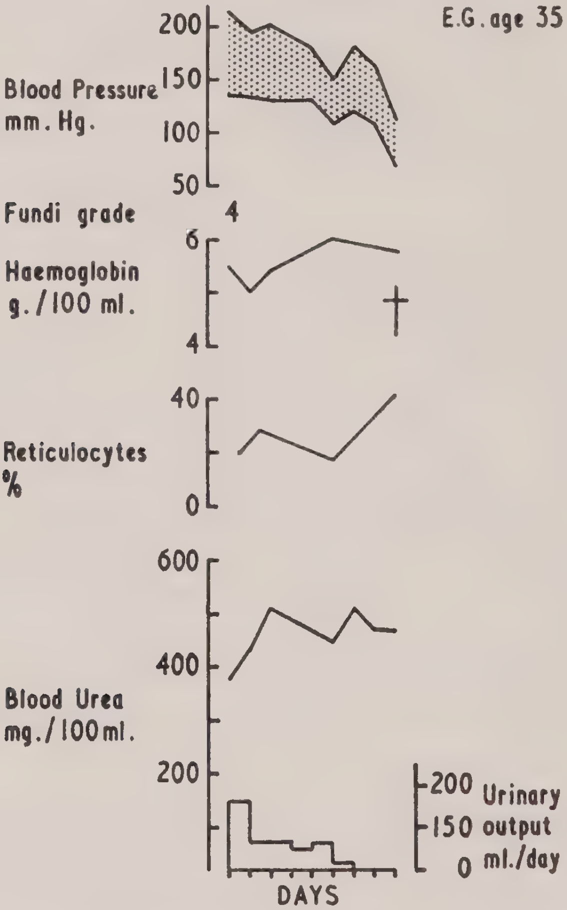

Fig. 221. Diagram illustrating the clinical course of, and laboratory findings in, a male patient who died of malignant hypertension (Case 27 of Dacie, 1954).

Eclampsia, Pre-Eclampsia and Puerperal Hæmolytic-Uræmic Syndromes. It has long been known that hæmoglobinæmia and hæmoglobinuria may be found in the acute phase of eclampsia. Young (1942) referred to a number of records in the German literature of the previous 60 years. Thus Albers (1938) described a patient who developed severe hypertension in the 9th month of pregnancy; the hæmoglobin fell to 42%, the reticulocyte count was 6·5% and microspherocytes and increased fragility were recorded. More recently, the subject has again received attention. A single case report was published by Kistner and Assali (1950) and a series of patients were investigated by Pritchard, Weisman, Ratnoff and Vosburgh (1953, 1954).

Pritchard and co-workers (1953) studied eleven eclamptic women and found some evidence of increased hæmolysis and/or hæmorrhagic phenomena in all of them. Three patients suffered from transient gross hæmoglobinæmia and hæmoglobinuria, and in six others a hæmolytic process was strongly suspected. In two patients erythrophagocytosis was observed in buffy-coat preparations of peripheral blood and the erythrocyte osmotic fragility was slightly increased. In two patients, too, the direct antiglobulin test was found to be transiently positive. No detailed studies on erythrocyte morphology were carried out.

A further, and fatal, case was described by Baker and colleagues (1953): acute hæmolysis with hæmoglobinæmia and hæmoglobinuria developed 24 hours *post partum* in a patient who was considered to have severe pre-eclampsia. Again, the blood picture was not described in detail.

Goodall (1961) published an interesting paper in which he described the case history of a woman who died of eclampsia. Hæmoglobinuria and hæmoglobinæmia were striking features. (The blood film was reported as showing no noteworthy features—although scanty normoblasts and myelocytes were seen in buffy-coat films.) At necropsy, areas of widespread hæmorrhage were seen in the liver, and this had led to extensive necrosis of parenchymal cells; much of the blood extraversated in the liver was lysed. Goodall suggested that the hæmoglobinæmia and hæmoglobinuria were probably the consequence of the breakdown of the large volume of blood which had been extraversated in the liver. He pointed out that a similar explanation had been put forward by Zangemeister as long ago as 1903!

Dacie (1954, Case 25, p. 368) described a patient on whom Cæsarean section had been performed because of severe pre-eclamptic toxæmia. Her fatal illness is illustrated in Fig. 222. Severe hæmolysis first occurred at a time when renal failure was only moderate in degree. Later, azotæmia became severe. Necropsy showed the changes of malignant nephrosclerosis, a striking feature being the intense fibrinoid necrosis of afferent glomerular arterioles.

The blood picture was typical of a microangiopathic hæmolytic anæmia of a moderately severe grade (Fig. 224).

Seftel and Metz (1957) were the next to stress how unusual the blood picture might be in eclampsia. They described two patients who developed severe hypertension and hæmolytic anæmia; and deformed erythrocytes, spherocytes, triangular and helmet cells and schistocytes were described as being present in their blood films. Later, MacWhinney, Packer, Miller and Greendyke (1962), in a discussion on thrombotic thrombocytopenic purpura, pointed out the similarities

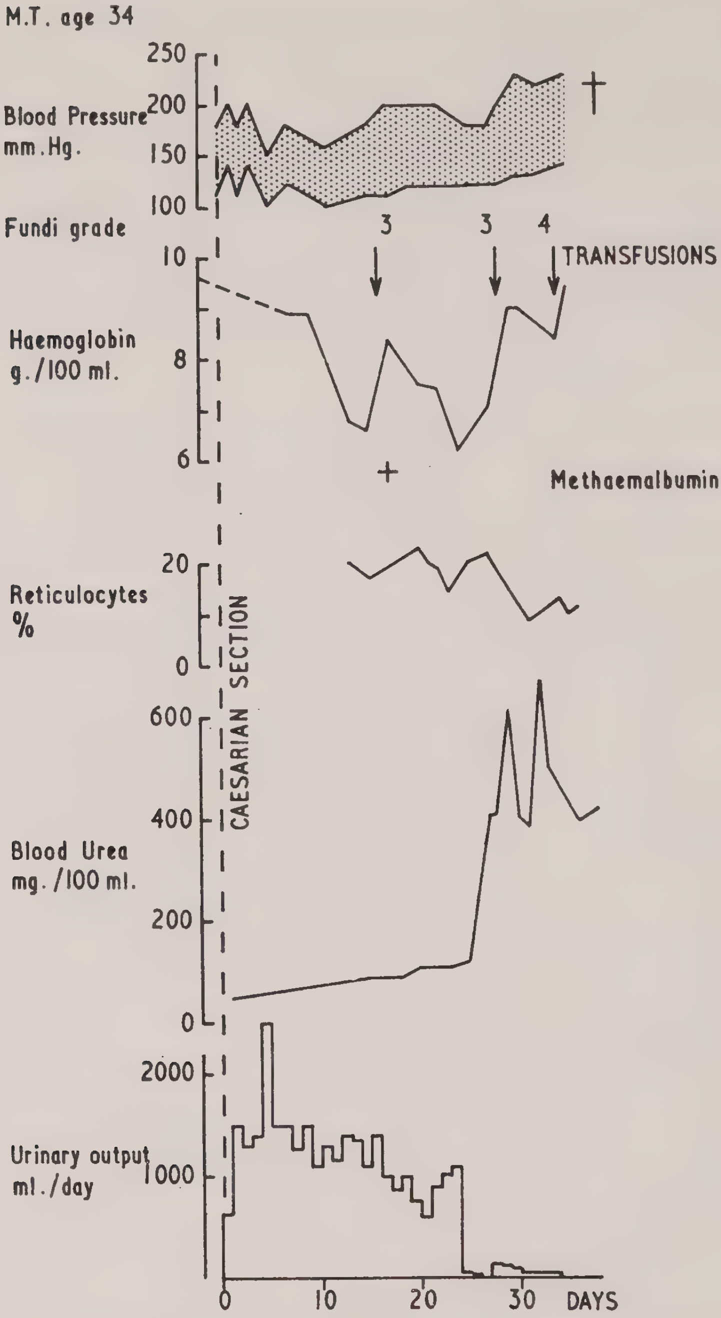

FIG. 222. Diagram illustrating the clinical course of, and laboratory findings in, a patient who died of malignant hypertension supervening on pre-eclampsia (Case 25 of Dacie, 1954).

between the blood picture in TTP and that in eclampsia—and also the similarities between the two disorders with respect to vascular lesions. They reproduced a film of a case of eclampsia which showed all the features of microangiopathic hæmolytic anæmia.

More recently, a further case (of fulminating eclampsia associated with intravascular hæmolysis and a hæmorrhagic diathesis) in which "burr" cells and triangular cells were seen in blood films was described by van Os and co-workers (1965).

Brain (1963) in his Thesis described the occurrence of microangiopathic hæmolytic anæmia in five women after childbirth.

(The first patient had been previously described by Dacie (1954).) The case histories of the other four women were in general similar. All became ill and anæmic between 3 and 9 days of delivery. In two, pre-eclamptic toxæmia was followed by a progressive rise in blood pressure and the development of signs and symptoms of malignant hypertension from which they died: in the other two patients the hypertension could be controlled but they became oliguric and died of renal failure despite repeated hæmodialyses.

At necropsy, fibrinoid necrosis of arterioles was associated with partial infarction or cellular proliferation of glomeruli, and in two cases patchy or extensive renal cortical necrosis, respectively.

A brief account of one of these patients is given below (Case 33) and the blood films of three of the women are illustrated in Figs. 224–226.

Case Report: Microangiopathic Hæmolytic Anæmia in Malignant Hypertension Supervening on Pre-Eclampsia

Case 33. The patient,[1] R. R., aged 27, was found in the 31st week of her second pregnancy to have a blood pressure of 170/125. She complained of severe headache and had œdema of the face and legs and heavy albuminuria. The ocular fundi were, however, normal. Two days after admission a surgical induction of labour was performed and she was delivered of a stillborn child on the 19th April 1953. The placenta was grossly infarcted but blood loss was not excessive.

Delivery was followed by diuresis but severe hypertension persisted and she became progressively more anæmic; her general condition also deteriorated, despite a series of blood transfusions. Exudates and hæmorrhages appeared in the ocular fundi and the heart enlarged; urine output diminished and the blood urea rose and eventually reached 476 mg. per 100 ml. She died on the 9th June 1963 after an illness of a little more than 2 months' duration (Fig. 227).

Laboratory Findings. Early in April the hæmoglobin was 11 g. per 100 ml. but by the 28th April, 9 days after delivery, it had fallen to 6·1 g. per 100 ml. Soon after this the blood picture was found to be typical of microangiopathic hæmolytic anæmia: stained films showed a moderate degree of anisocytosis and polychromasia as well as fair numbers of contracted and distorted cells (Fig. 225); the reticulocyte count was 9%. Platelets were present in stained films in moderate numbers.

The direct antiglobulin test was negative and osmotic fragility was normal. The life-span of normal transfused erythrocytes was estimated by the Ashby method to be 16 days (Prof. P. L. Mollison). A few days before death the total leucocyte count was 30,000 per cu. mm., with 88% neutrophils, and the reticulocyte count was 13%.

Necropsy Findings. The main findings were found to be those of malignant nephrosclerosis. The kidneys showed numerous small areas

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[1] A more complete clinical and pathological account of this patient was given by Counihan and Doniach (1954, Case 2).

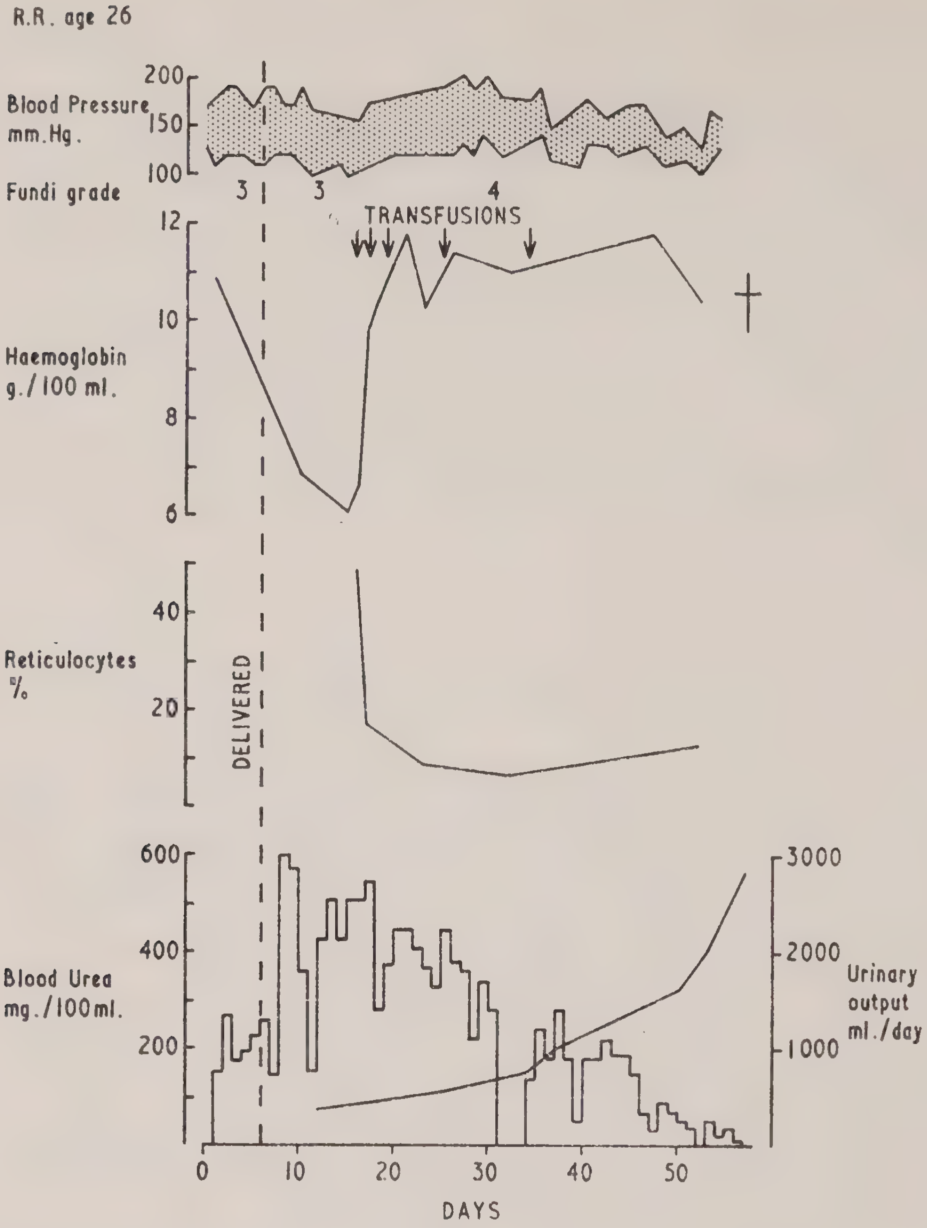

FIG. 227. Diagram illustrating the clinical course of, and laboratory findings in, a patient who died of malignant hypertension following pre-eclampsia (Case 33).

of focal cortical necrosis which were associated with fibrinoid necrosis and thrombosis of interlobular arteries. Many of the afferent arterioles, too, showed fibrinoid necrosis of their walls and their associated glomeruli partial infarction or proliferation of endothelium. Vascular changes, *i.e.*, fibrinoid necrosis and occlusive thrombi, were found in scattered arterioles in the uterus, ovaries and rectum. The Kupffer cells of the liver and the siderophages in the spleen contained an excess of iron.

Summary. The patient died of renal failure due to malignant nephrosclerosis, after a short illness resembling pre-eclampsia. This was associated with a marked anæmia of the microangiopathic hæmolytic type.

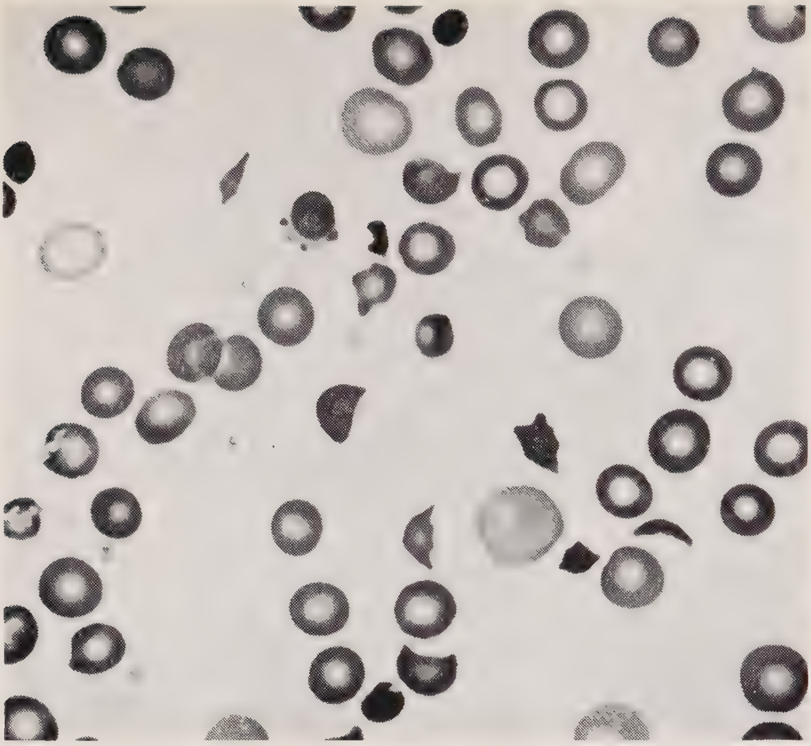

FIG. 223. Photomicrograph of a blood film of a male patient who died of malignant hypertension complicated by hæmolytic anæmia (Case 27 of Dacie, 1954). There is marked anisocytosis and macrocytosis and many irregularly contracted cells and cell fragments are also present. × 700.

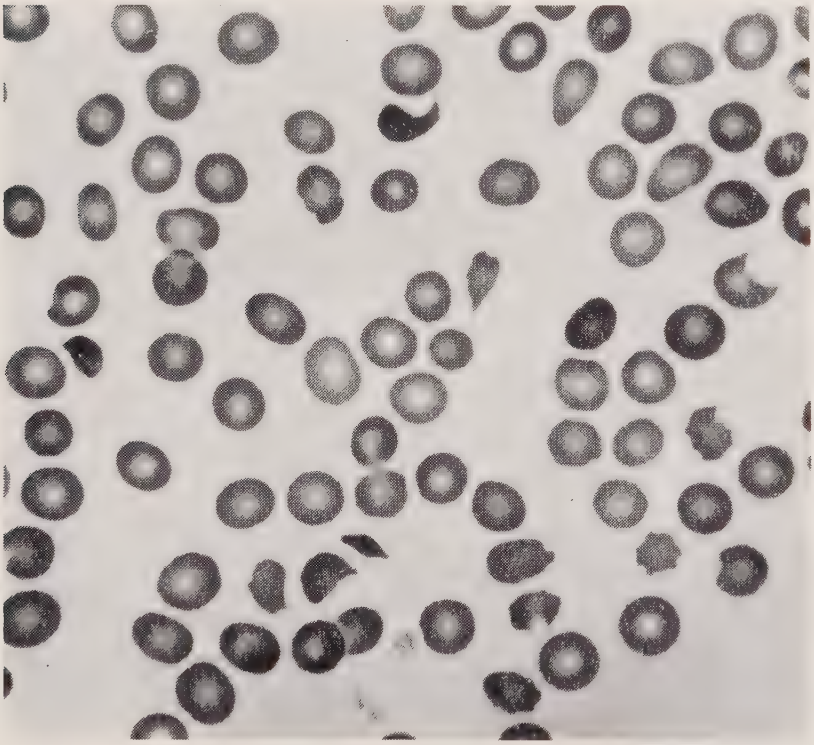

FIG. 224. Photomicrograph of a blood film of a patient who died of malignant hypertension following pre-eclampsia (Case 25 of Dacie, 1954). There is moderate anisocytosis and some irregularly-shaped contracted cells are present. × 700.

To face p. 880.

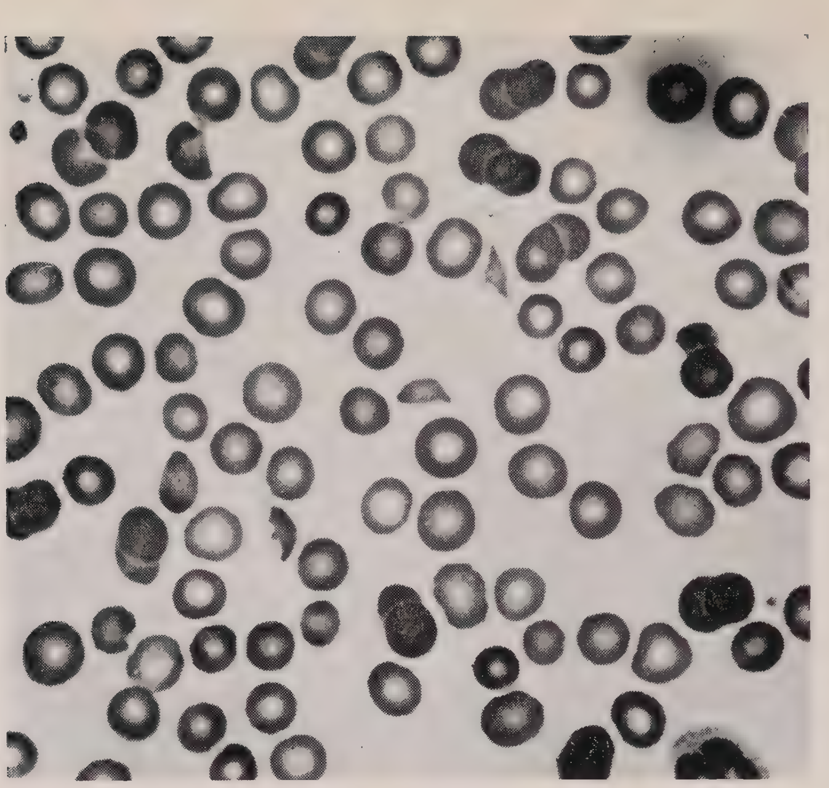

FIG. 225. Photomicrograph of a blood film of a patient who died of malignant hypertension following pre-eclampsia (Case 33). There is slight anisocytosis, a tendency to microcytosis and occasional contracted and distorted cells and "burr" cells are present. ×700.

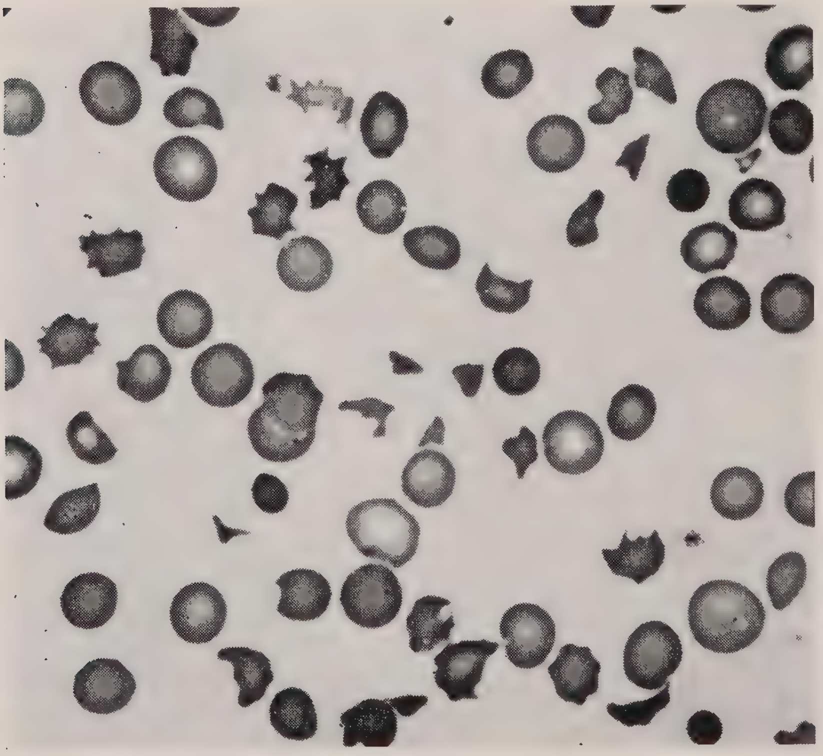

FIG. 226. Photomicrographs of a blood film of a patient (V. M.) who died of pre-eclampsia and patchy renal cortical necrosis. Fibrinoid necrosis was found in renal arterioles as well as in arterioles in a lymph node and in the pancreas.

There is marked anisocytosis and many irregularly contracted cells and cell fragments are present, including triangular forms. There is also some crenation. ×700.

MICROANGIOPATHIC HÆMOLYTIC ANÆMIA

The term *microangiopathic hæmolytic anæmia* has been used on several occasions in the preceding pages; it was introduced by Brain, Dacie and Hourihane (1962) to describe an unusual type of hæmolytic anæmia found in association with thrombotic thrombocytopenic purpura, "acute glomerulonephritis" in infancy and childhood (the hæmolytic-uræmic syndrome), renal cortical necrosis, microscopic polyarteritis nodosa, some cases of malignant hypertension, and in carcinomatosis. In all the above disease processes thrombi occur within arterioles and capillaries with or without fibrinoid necrosis of arterioles and necrotizing arteritis, or, in the case of carcinoma, the tumour has been found to be widely disseminated and, in some cases at least, small blood vessels have been invaded by tumour cells.

The blood pictures in each of the above conditions are strikingly similar. Brain, Dacie and Hourihane's main thesis was that the changes had in all probability a common origin; namely, that the erythrocyte damage leading to an increased rate of hæmolysis was brought about in some way by close contact between the cells and the abnormal diseased environment through which they circulated.

It must be emphasized that microangiopathic hæmolytic anæmia was not looked upon by Brain, Dacie and Hourihane as a "new" disease or indeed as a disease *per se*: moreover, it was not claimed that the blood picture had not been described previously. It certainly had, but under a variety of names, and without any unifying concept. Some of the more important publications in which the descriptions of the blood picture exactly fits that of microangiopathic hæmolytic anæmia are described below in chronological order: Adelson and Stefanini (1952) and Adelson, Heitzman and Fennessey (1954) (macrocytosis, spherocytosis, target cells and helmet cells), Dacie and his co-workers (1953) ("contracted and distorted microcytes with angular outlines, many being almost triangular in shape"), Dacie (1954, p. 13) ("irregular crenation and contraction"), Gasser and his colleagues (1955) (spherocytosis and disintegrating ("*zerfallende*") forms), Allison (1957) (poikilocytosis, fragmentation and severe distortion), Aherne (1957) (burr (uræmic) poikilocytes (crescentic in shape)) and Lock and Dormandy (1961) (red-cell fragmentation syndrome).

Brain, Dacie and Hourihane (1962) were able to show that the peculiar erythrocyte contraction, distortion and fragmentation was not related to the degree of azotæmia from which the patient might be suffering (Fig. 218, p. 870); on the other hand, there appeared to be a definite link between the presence of widespread disease affecting small blood vessels and a markedly abnormal blood picture (Fig. 228). The patients, too, who were most anæmic and were suspected of suffering from the most severe hæmolysis had, in general, as might be expected, the most abnormal films (Fig. 229). However, there was no correlation between the hæmoglobin level on admission and the degree of azotæmia (Fig. 230).

Some representative films are shown in Figs. 207–210, 215, 216 and 223–226. Some of the contracted erythrocytes are round or almost round in contour and these can correctly be referred to as spherocytes, but the typical finding is for the contracted erythrocytes to be irregular in shape rather than round and for some of the cells to be roughly triangular or helmet-shaped or to be irregularly shaped and to show one or more sharp spines ("burr cells" (see below)). The majority of the abnormally shaped cells have clearly undergone contraction and distortion, and have not actually fragmented but it is characteristic to find some densely- (or palely-) staining very small cells which can only be looked upon as cell fragments (schistocytes). Some patients' films show superadded crenation and this leads to very abnormally shaped cells (Figs. 208 and 226).

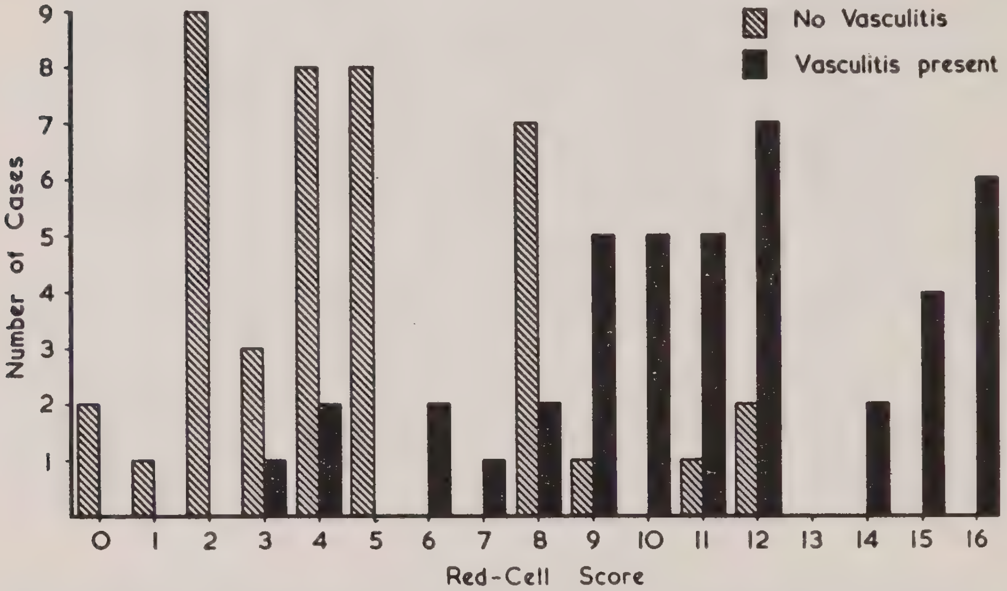

FIG. 228. Relationship between the degree of erythrocyte abnormality (score—abscissa) and the presence or absence of vascular lesions in 115 patients. Cross-hatched columns, vascular lesions *not* present; black columns, vascular lesions present. (From Brain, Dacie and Hourihane (1962).)

It must be emphasized that the above-described changes are not artefacts which have developed *in vitro*: they can be seen in "wet" preparations of blood and it is reasonable to assume that the abnormal cells are present in circulating blood. It has been claimed, too, that they can be seen in histological sections (see p. 869); here, however, the possibility of artefact, post-mortem or otherwise, is not easy to exclude.

Erythrocytes showing diffuse basophilia (reticulocytes) are usually perfectly round and undistorted and being macrocytes they contrast vividly in films with the more mature cells which show all grades of distortion and variation in shape. (This observation fits in well with the concept that the shape change is acquired after the cells leave the bone marrow and not the consequence of dyshæmopoiesis.) The reticulocyte count may reach 30% or more, even in the presence of severe azotæmia. Autoagglutination is not a feature of the blood film in microangiopathic hæmolytic anæmia; rouleaux formation is, however, often marked.

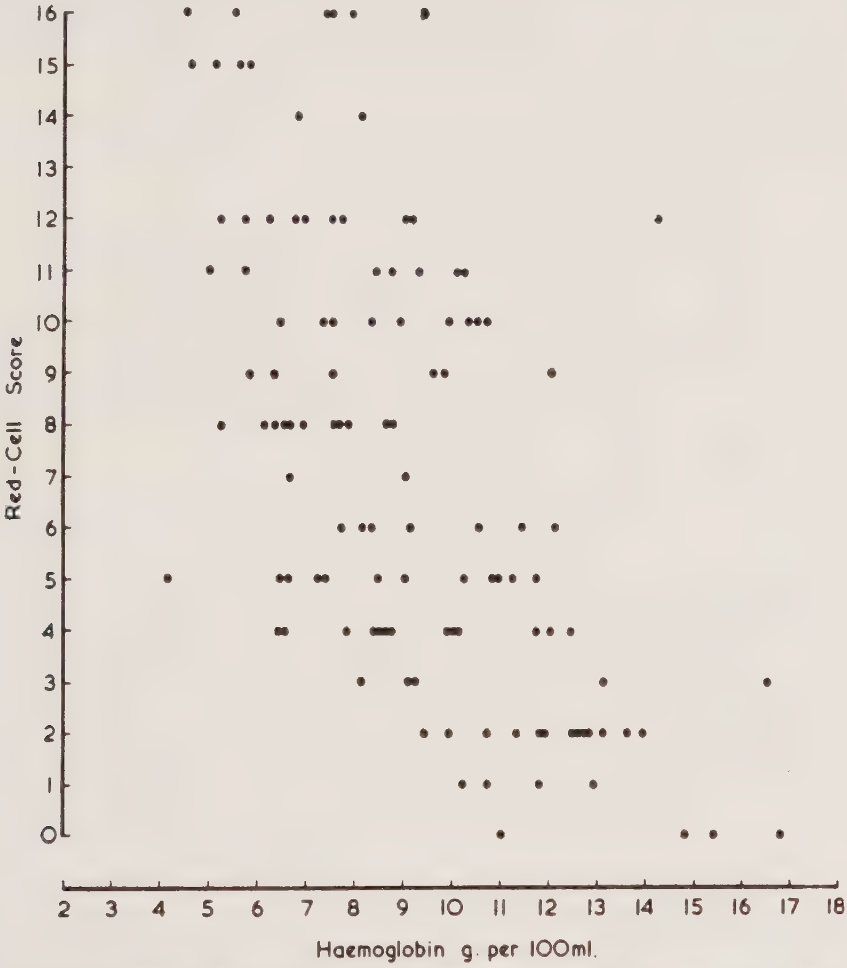

Fig. 229. Relationship between erythrocyte abnormality (score—ordinate) and hæmoglobin level in 115 patients. (From Brain, Dacie and Hourihane (1962).)

The changes in leucocyte and platelet count—typically neutrophilia with a shift to the left and thrombocytopenia—which accompany those of microangiopathic hæmolytic anæmia have already been mentioned (p. 861).

"Burr" cells. Schwartz and Motto (1949) described as a "burr" cell, "a mature erythrocyte about 7·5 micra in diameter, which had one to

several spiny projections along its periphery." Such cells were reported to be present in small numbers (maximum 3·7%) in patients with a variety of blood diseases, and also in uræmia, carcinoma of the stomach and bleeding peptic ulcer; they were found to be absent or very rare in the blood of normal people. Burr cells are but one of the abnormal cell types seen in microangiopathic hæmolytic anæmia (Figs. 208 and 226). Schwartz and Motto distinguished between burr cells and crenated cells; however, in microangiopathic hæmolytic anæmia burr cells appear to be most frequent when the erythrocytes as a whole show an increased tendency to crenation.

As already stressed, the presence of burr cells in the circulating blood (and other types of erythrocyte distortion) is not directly related to the degree of azotæmia, if any, present; but their presence should certainly alert the investigator to the possibility of renal failure (Aherne, 1957; Marmont, Bellotti and Azzena, 1959, etc.).

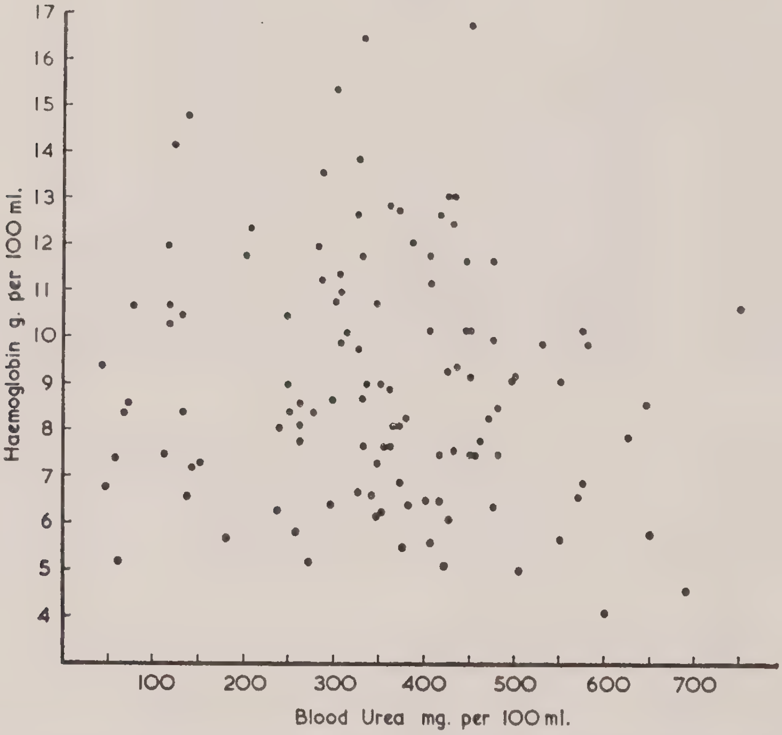

FIG. 230. Relationship between hæmoglobin level and blood urea on admission in a series of 115 patients, 113 of whom had been admitted to hospital with acute or chronic renal failure. (Unpublished data of Brain, Dacie and Hourihane (1962).)

How burr cells are formed has been a matter of speculation. Bell (1963) published a series of photographs which suggested that they resulted from a vacuole, developing at the periphery of the cell, which subsequently enlarged and finally ruptured, leaving an abnormally shaped erythrocyte with fine projections extending from the shoulders of the defect. It is possible in reality that some at least of the "vacuoles" are actually areas of extreme thinning of the cells, where the two surfaces have come in contact. Rupture of the membrane at this point would lead, as Bell suggested, to very abnormally shaped cells. These suggestions leave unanswered, unfortunately, the cause of the development of the vacuoles or areas of thinning and fail to explain the mechanisms which lead to the irregular contraction and other types of cell distortion so typical of microangiopathic hæmolytic anæmia.

Other Hœmatological Findings. Mention has already been made of the increased osmotic and mechanical fragilities and the rises in plasma hæmoglobin and other biochemical changes seen in association with thrombotic thrombocytopenic purpura (p. 861). Exactly the same changes seem to take place in the microangiopathic hæmolytic anæmia occurring in the hæmolytic-uræmic syndrome in infants and children, and in malignant hypertension, etc.

PATHOGENESIS OF HÆMOLYSIS IN RENAL FAILURE

It is not easy to present a clear picture of the mechanism(s) which give rise to excess hæmolysis in renal failure. Two main subjects will, however, be discussed: the role of renal failure (azotæmia) *per se*, and the role of vascular disease, which is in many cases the basis for the renal failure.

The Role of Azotæmia

Hæmolysis following Nephrectomy in Animals. The development of techniques making it possible to maintain life for some days after bilateral nephrectomy has allowed erythrocyte life-span and other studies to be carried out experimentally on animals. Muirhead, Jones and Grollman (1952), Muirhead, Jones, Stirman and Lesch (1953) and Muirhead and Jones (1958) obtained evidence pointing to increased hæmolysis in rabbits and dogs, respectively, submitted to bilateral nephrectomy. Erythrophagocytosis, particularly in lymph nodes, was demonstrated. Houck (1954) found that the anæmia of bilaterally nephrectomized dogs was not affected by prior splenectomy. Muirhead and Jones (1958) demonstrated that biochemical changes in erythrocytes (lowered glucose utilization and phosphate uptake and reduced glutathione) occurred within 24 hours and they interpreted the changes as indicating rapidly developing injury. In a later paper (Muirhead and Jones, 1963) the protective effect of auto-transplantation of renal medulla and other evidence were held to indicate that a renal medullary factor normally protected erythrocytes in some way against hæmolysis.

Hæmolysis in the Anephric Man. The development of successful techniques of renal dialysis has allowed bilateral nephrectomy to be carried out in man also, and opportunity has been taken to study erythropoiesis and erythrocyte destruction in certain cases. Nathan, Schupak, Stohlman and Merrill (1964), who investigated five patients, found moderate increases in hæmolysis in four of them (^{51}Cr T$\frac{1}{2}$, 25, 13, 14, 13 and 16 days, respectively). It should be stressed that in these patients—as opposed to the experimental animal—the azotæmia was being controlled, as far as was practicable, by intermittent dialysis.

Erythrocyte Metabolism in Renal Failure in Man. This subject has been explored in recent years by several groups of workers. Rees and his co-workers (1957) studied 10 chronically uræmic patients—all showed increased erythrocyte destruction. *In vitro*, synthesis of phosphate esters and potassium flux could be maintained when uræmic cells were incubated in fresh normal plasma fortified with inosine, but in the patients' own plasma both measurements of metabolic activity were subnormal Hurt and Chanutin (1964), who carried out rather similar studies in 23 patients, obtained inconsistent results, which they considered might reflect plasma changes, in inorganic phosphate for instance, rather than intrinsic abnormalities of erythrocyte metabolism. 2,3-DPG concentrations were low in three patients but higher than normal in 14, while ATP values were high in about half the patients.

Theil, Brodine and Doolan (1961) investigated another aspect of erythrocyte metabolism. Reduced glutathione was found to be substantially lowered in concentration, and its stability moderately impaired, in a group of patients with acute renal failure; and in chronic renal failure, despite a normal mean level, there was some impairment in stability.

An abnormality in ion transport in the erythrocytes of uræmic patients was reported by Welt, Sachs and McManus (1964). In some patients a higher than normal concentration of sodium was found and this was attributed to diminished pump activity. The ATP levels were, nevertheless, often supranormal, and the impaired pump activity was attributed to a lowered activity of the alkali-metal-sensitive component of ATPase.

If the erythrocyte in uræmia is metabolically abnormal, as the above-mentioned studies suggest, then this might be expected to be manifested by an accelerated rate of autohæmolysis *in vitro*. A small increase has in fact been demonstrated by Balestri, Cioni and Giovannetti (1965): in 28 chronically uræmic patients the mean autohæmolysis at 48 hours was 1·78%, compared with a normal mean of 0·81%. Cioni, Balestri and Giovannetti (1965) carried out some crossed-incubation experiments and concluded that the increased autohæmolysis depends, at least in part, on the presence of an extracorpuscular factor which is dialysable. They showed, too, that the increased autohæmolysis was paralleled by an increased loss of potassium.

The Role of Vascular Disease

The author feels that all the evidence points strongly in favour of the severe hæmolysis which may accompany acute renal failure as being a consequence of vascular disease rather than of azotæmia. Not only is it a fact that severe grades of hæmolysis rarely, if ever, occur in chronic renal failure, even if azotæmia is severe, but in patients who have acute renal failure the degree of azotæmia and the extent to which the patients' erythrocytes are abnormal are not closely correlated (Fig. 218, p. 870). There is, on the other hand, a correlation between the severity of the abnormality in blood picture and the occurrence of vascular disease (Fig. 228).

How disease of small blood vessels could bring about hæmolysis is an interesting question. The contraction and distortion of erythrocytes, and evidence of actual fragmentation, too, point strongly to the cells being severely damaged, and Brain, Dacie and Hourihane (1962) suggested that this could be brought about locally by physico-chemical or even mechanical trauma occurring while the cells were circulating through diseased vessels. If histological sections are examined under high magnification, erythrocytes can be seen within intraluminal thrombi or sometimes infiltrating the walls of diseased vessels. It certainly seems possible that intimate contact with necrotizing tissue could seriously damage the erythrocytes and that after many circulations through, and exposures to, partially obstructed vessels and their affected walls the cumulative effect of the damage might be such as to lead to lysis locally or in the general blood stream as the result of mechanical stresses or in organs rich in phagocytic cells such as the spleen.

If the actual mechanism of erythrocyte damage is a chemical one —as appears most likely—it has to be admitted that the nature of the damaging material(s) is as yet unknown. Proteolytic or lipolytic enzymes are probably liberated by any tissue undergoing necrosis (see p. 793), and they could be responsible; alternatively, or in addition, enzyme activity triggered off by intravascular fibrin deposition could be responsible.

Another possibility is that the erythrocytes are damaged by becoming attached to fibrin-covered endothelium, with rupture of the membrane, release of hæmoglobin, and possibly the formation of cell fragments or microspherocytes (Brain, Esterly and Beck, 1967). Fibrin has in fact been seen on or close to the surface of erythrocytes in thrombosis *in vivo* (Marr, Barboriak and Johnson, 1965) and tears in the membrane and loss of hæmoglobin have been reported to occur under these circumstances (Pederson, Marr, Tebo and Johnson, 1965).

A further but perhaps less likely possibility is that mechanical stresses alone, unconnected with attachment of erythrocytes to fibrin-covered endothelium, could produce intravascular hæmolysis in small blood vessel disease as they have been found to after certain operations on the human heart (see p. 963). It is conceivable that a sudden reduction in the lumina of affected small vessels could lead to erythrocyte damage and ultimately to hæmolysis.

Experimentally, Brain (1963), Brain and Beck (1965) and Brain, Esterly and Beck (1967) have demonstrated that intravascular coagulation brought about by the Shwartzman reaction or by the infusion of thrombin is associated in the rabbit with intravascular hæmolysis. Brain, Esterly and Beck reported that the hæmoglobinæmia so produced was not dependent upon the presence of the spleen or kidneys or leucocytes but could be prevented by heparin. Fragmented erythrocytes appeared in the circulation and it was suggested that the hæmoglobinæmia was due to the intravascular destruction of erythrocytes at the site of the fibrin thrombi, and also that alteration in the erythrocyte surface might influence blood flow through affected capillaries and promote thrombus formation, thus creating a vicious circle. These rather exciting experimental studies in animals of the interrelationships between thrombosis *in vivo* and intravascular hæmolysis seem to provide a close parallel to the disorders occurring spontaneously in man in which hæmolytic anæmia and thrombocytopenia accompany thrombotic disease affecting small blood vessels.

"*Minor Forms of Microangiopathic Hæmolytic Anæmia*"

Brain, Dacie and Hourihane (1962) in their review of more than 100 films from patients suffering from acute or chronic renal failure found all grades of blood pictures—from films which were grossly "microangiopathic" to films from patients not suffering from overt hæmolytic anæmia (containing only a few abnormal cells) which were hardly distinguishable from normal. To what extent slightly abnormal or "intermediate" blood films are produced by the same mechanism—contact with abnormal small blood vessels—as is thought to operate in severely affected patients is a matter of speculation. As, however, the afferent blood vessels or the capillaries of the glomeruli are involved in most types of renal disease, the hypothesis of damaging contact with abnormal small blood vessels could provide at least a part explanation for the minor degrees of increased hæmolysis seen in most cases of far advanced chronic renal disease.

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CHAPTER 15

SECONDARY OR SYMPTOMATIC HÆMOLYTIC ANÆMIAS: III MISCELLANEOUS CONDITIONS

HÆMOLYTIC ANÆMIA IN ACUTE AND CHRONIC BACTERIAL INFECTIONS

Introduction

Anæmia is a common accompaniment of bacterial and viral diseases, and in this section short accounts will be given of the role played by hæmolysis in its causation in infections of bacterial origin. (The hæmolytic anæmias associated with viral infections have already been dealt with in Chapter 9, Part II.)

The possible ways in which infection may lead to increased hæmolysis are considered on p. 913, after brief discussions on the incidence of overt or latent hæmolysis in some of the main types of infection. A few general points may, however, be made at this stage:

1. The anæmia of infection has without doubt a complicated origin; increased hæmolysis is but one possible mechanism and, if cases of actual bacterial invasion of the blood stream are excluded, not necessarily a very important one (Cartwright and Wintrobe, 1952). More often than not hæmolysis is occult rather than overt.

2. Infection may cause, perhaps always causes, an exacerbation of a previously existing chronic hæmolytic anæmia (Wright and Gardner, 1960); it may even lead to the disclosure and diagnosis of a hitherto unsuspected hereditary hæmolytic disorder (*e.g.*, Case 5 of Dacie (1954, p. 99); Danon, De Vries, Djaldetti and Kirschmann (1962)).

3. It is possible that certain people develop increased hæmolysis when suffering from infections more readily than others. The mechanism of this is generally unknown but in a few individuals it may be based on a deficiency of the erythrocyte enzyme glucose-6-phosphate dehydrogenase (see Chapter 16, Part IV).

REPORTS AND STUDIES ON OCCULT HÆMOLYSIS OR OVERT HÆMOLYTIC ANÆMIA IN BACTERIAL INFECTIONS

Coccal Infections: Septicæmia and Endocarditis

Relatively few detailed studies seem to have been made. However, clinical and/or laboratory evidence for increased hæmolysis has been reported in: *streptococcal septicæmia* (Mollison, 1947); *streptococcal* or *staphylococcal endocarditis* or *pneumococcal pyœmia* (Gasser, 1951); *pneumococcal pyœmia* (Brown, 1951); *anœrobic streptococcal sepsis* (Gerversman and Go Gien Hoo, 1951); *bacterial endocarditis* (Schlegel and Böttner, 1951; Daland, Gottlieb, Wallerstein and Castle, 1956); *subacute bacterial endocarditis* (Grobbelaar, 1958; Hennemann, 1959; Jandl, Jacob and Daland, 1961).

The interesting occurrence of transient hæmolytic anæmia and thrombocytopenia was described by Nussbaum and Dameshek (1957) in a patient subsequently found to have a meningococcal infection. A tentative diagnosis of thrombohæmolytic thrombocytopenic purpura (TTP) had first been considered, and Nussbaum and Dameshek pointed out that experimentally in rabbits lesions in small blood vessels had been produced resembling those of TTP following the injection of meningococcal endotoxins (Brunson, Thomas and Gamble, 1955). In this connection the present author has seen a blood picture very like that of microangiopathic hæmolytic anæmia in a child suffering from meningococcæmia (Fig. 231).

Hæmophilus influenzæ Infections: Meningitis, etc.

Hagberg (1951–2) described the shortened survival of normal erythrocytes transfused to a child suffering from *Hæmophilus influenzœ* meningitis. Margolis (1955), in a comprehensive and interesting study of children with meningitis due to *Hæmophilus influenzœ*, concluded that the hæmolysis observed was largely due to the presence of anti-human erythrocyte antibodies at high titres in rabbit immune sera given in treatment: he showed that anæmia was less severe and that spherocytosis did not develop in children who had not been treated with the rabbit serum or who had been given the serum after the anti-human antibodies it contained had been absorbed.

Infection with Enterobacteria: Salmonella typhi; Salmonella sp.; Escherichia coli, etc.

Salm. typhi. A hæmolytic episode is a well known although uncommon complication of typhoid. However, although hæmoglobinuria had been recognized by Osler in a single case as far back as 1895, it is only in the last 20 years or so that much attention has been paid to the occurrence of hæmolytic anæmia.

In 1945 Berman, Braun and Rachmilewitz concluded that hæmolysis rather than hæmorrhage was the more likely cause of the mild to severe anæmia associated with reticulocyte counts of 4·6–10% which had developed, usually in the second or third week of illness, in nine out of a series of 152 patients with typhoid. Evidence for the occurrence of indisputable hæmolysis, *i.e.*, anæmia accompanied by hæmoglobinuria, was reported by Shaw (1951), Gordon Smith (1951), McFadzean and Choa (1953) and Ruggieri (1961).

Grobbelaar (1958) described three patients with typhoid in whom there was suggestive evidence of hæmolysis; their erythrocytes gave positive direct antiglobulin tests. Grobbelaar obtained positive antiglobulin tests, too, with 30 out of 100 blood samples sent to the laboratory from patients suspected of having typhoid. He considered that in South Africa typhoid was second only to the malignant reticuloses as a cause of symptomatic auto-immune hæmolytic anæmia.

A further interesting account has more recently also been reported from South Africa. Retief and Hofmeyr (1965) described the occurrence of severe anæmia and hæmoglobinuria as the dominant clinical finding in a febrile male Bantu aged 36 (he also had melæna). Spherocytes, triangular cells and fragmented cells were recognized in the blood film. The direct antiglobulin test was negative and the blood urea was 16 mg. per 100 ml. initially (it rose to 104 mg. later). *Salm. typhi* was isolated three times by blood culture. Both the hæmolysis and the pyrexia subsided within a few days of starting treatment with Chloromycetin. Retief and Hofmeyr, while stating that less than 40 authentic cases of acute hæmolytic anæmia had been reported in typhoid, concluded, nevertheless, that in at least 50% of cases evidence of intravascular hæmolysis, *i.e.*, hæmoglobinæmia, would be forthcoming if looked for in the first week at the time of the bacteræmia.

Other Salmonellæ. Davidson and Fullerton (1958) reported acute hæmolytic anæmia in a patient infected with *Salmonella* Dublin, and the present author is aware of the occurrence of acute hæmolytic anæmia in a boy a week or so following infection with *Salmonella œrtrycke.*[1]

Escherichia coli. Horowitz, Javid and Spaet (1960) described the occurrence of "Lederer's anæmia" in a small girl who was suffering from an *E. coli* infection of the urinary tract. The hæmolysis subsided when the infection responded to treatment with chloramphenicol.

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[1] This boy later developed the symptoms and signs of Wilson's disease (see p. 833).

Mycobacterium tuberculosis

The association between tuberculosis and increased hæmolysis is a complicated one. On the one hand, there is the possibility that patients who have had overt tuberculosis may be more prone to develop "cryptogenic" hæmolytic anæmia than patients who have not had tuberculosis. Fisher (1947) for instance, in reviewing 11 miscellaneous cases of acquired hæmolytic anæmia, found that five of the patients had suffered from tuberculosis in the past. On the other hand, there are patients who develop severe hæmolytic anæmia and are then found to have tuberculosis at splenectomy or necropsy. Early examples of the latter association were reported by Thompson (1936) and Engelbreth-Holm (1938).

More recently, severe hæmolytic anæmia in patients with active tuberculosis has been described by the following authors and in the following circumstances: Spira (1943, *hilar tuberculosis* in childhood); Mollison (1947, *advanced pulmonary tuberculosis*); Lindeboom (1950, *areactive generalized tuberculosis*); Hemmeler and Lob (1950, *splenic tuberculosis*); Plauchu, Revol, Lejeune and Jouvenceaux (1952, *miliary tuberculosis*); Plum (1954, *generalized lymph-node tuberculosis in a child*); Guszich (1957, *splenic tuberculosis*), and Jandl, Jacob and Daland (1961, *miliary tuberculosis*).

Minor degrees of hæmolysis, based on erythrocyte survival studies, have been also reported by Schlegel and Böttner (1951) in pulmonary tuberculosis and by Miescher, Gsell and Fust (1955) in tuberculosis in guinea-pigs.

Vibrio choleræ

De, Sengupta and Chanda (1954) have reported the occurrence of intravascular hæmolysis in cholera.

Bartonella bacilliformis

Oroya fever (Carrion's disease), in which striking hæmolysis is associated with intra-erythrocytic parasitism with Bartonella, has a large literature. The clinical and hæmatological findings are well described in the reviews of Ricketts (1948, 1949) and Reynafarje and Ramos (1961).

Clostridium welchii

The α toxin of *Cl. welchii* acts as a lecithinase and severe or even fulminating hæmolysis is a well known consequence of *Cl. welchii* infection. The vast majority of infections in which hæmolysis has occurred have been post-abortal or puerperal, and it is an interesting fact that hæmolysis is not a frequent or dominant feature in surgical gas gangrene, although it has been reported (Clayton and Dacie, 1936). Severe hæmolysis has also been observed in acute cholecystitis when *Cl. welchii* has been the infecting organism (Bennett and Healey, 1963).

Post-Abortal and Puerperal Infections with *Cl. welchii*. There is a large literature, which dates back many years. Most, although not all, of the patients have quickly become severely ill, with rapidly darkening jaundice which in a few hours often has deepened to a bronze or even mahogany colour. Hæmolysis is essentially intravascular, and the serum is often deep red in colour. Hæmoglobinuria is characteristically intense but there is often anuria, and a hæmorrhagic state may develop as an additional complication. The mortality is high.

Hill (1936) reported on the early literature and described 30 cases seen in Australia. Most of the patients became markedly jaundiced and in many there was evidence of gross hæmolysis. Douglas, Carney and Pellillo (1953) also reviewed the problem and described six personally studied patients, in four of whom hæmolysis was a conspicuous feature. Hadley and Ekroth (1954) described two further cases and stressed that the occurrence of spherocytosis was a valuable pointer to *Cl. welchii* infection.

Mahn and Dantuono (1955), in a further large review, described their experience of 75 cases seen in a 5-year period in Santiago, Chile: they stressed the frequency with which a hepato-renal syndrome developed in patients who survived the initial acute hæmolytic phase and also the frequent occurrence of hæmorrhagic phenomena. More recent case reports include those of Isham and Finch (1956), Thompson and Nixon (1959), Davenport and Oken (1960) and Kadner and Anderson (1963).

Kadner and Anderson (1963) did not succeed in culturing *Cl. welchii* from all their cases and they made the point that infection with other organisms might produce a similar syndrome.

It should be added that a clinical syndrome and blood picture simulating that produced by *Cl. welchii* infection, although more benign, has followed injecting soapy water into the uterus in an attempt to produce abortion (Moulonguet, Rouillac and Mingaud, 1953).

Blood Picture in Cl. welchii Hæmolytic Anæmia

Intense hæmoglobinæmia, microspherocytosis and hyperleucocytosis with a shift to the left (a leukæmoid picture) are characteristic of *Cl. welchii* infection. Thrombocytopenia has often been

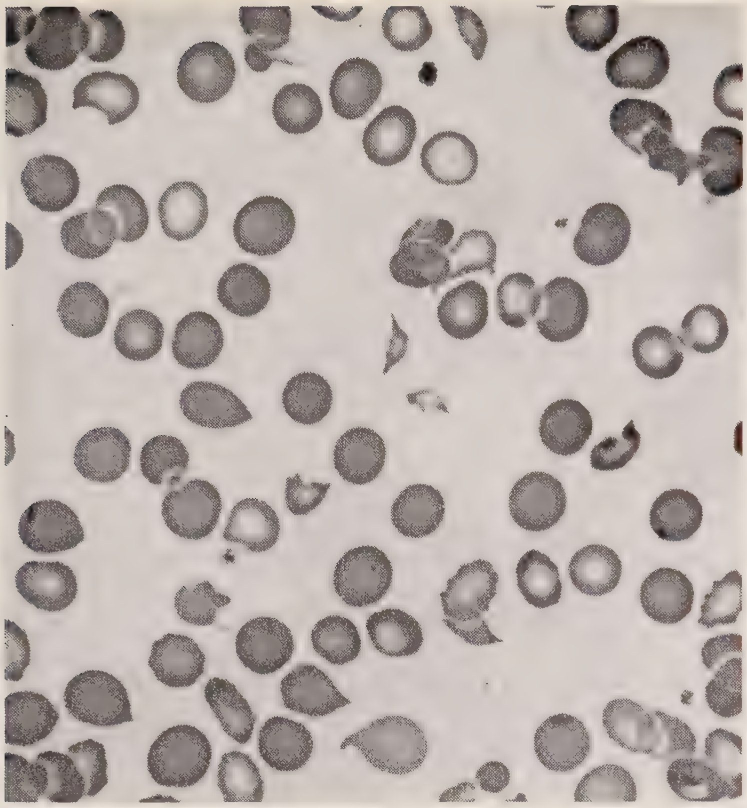

FIG. 231. Photomicrograph of a blood film of a child (G. A.) who developed meningococcæmia. There is considerable anisocytosis, anisochromasia and poikilocytosis, and contracted and distorted cells and cell fragments are also present. ×700.

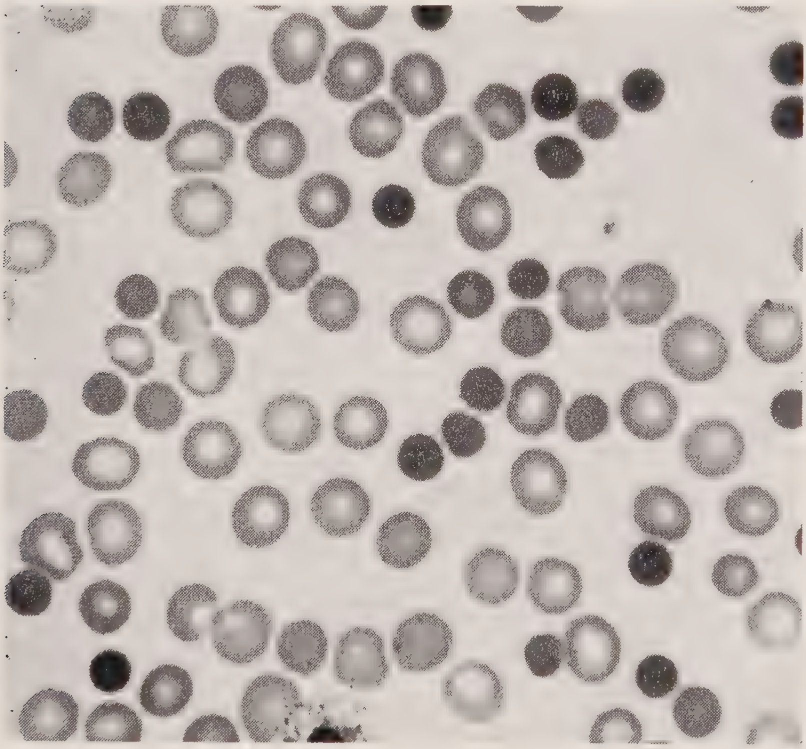

FIG. 232. Photomicrograph of a blood film of a woman (M. C.) who developed *Cl. welchii* septicæmia following an abortion. There is intense microspherocytosis. ×700.

reported, too (Mahn and Dantuono, 1955; Isham and Finch, 1956; Thompson and Nixon, 1959) (Fig. 232).

In the patient with gas gangrene following a leg injury reported by Clayton and Dacie (1936) the total leucocyte count was 87,000 per cu. mm., with 42% of neutrophil precursors present. More usually, the maximum leucocyte count is within the 30,000 to 60,000 per cu. mm. range. Spherocytosis is often intense in degree (Fig. 232) and osmotic fragility is correspondingly increased. Erythrophagocytosis may be conspicuous in buffy-coat preparations (Isham and Finch, 1956).

Pathogenesis of Increased Hæmolysis in Bacterial Infections

There are a number of possible ways in which infection could lead to increased erythrocyte destruction.

1. Direct Effect of Bacteria on Erythrocytes. First, there is invasion of erythrocytes by the parasite, as in Bartonella infection and malaria. Here one must suppose that interference with the cells' metabolism by the growth of the invading organism leads to premature senescence or, by altering in some way the cells' surface properties, to phagocytosis by macrophages.

Rudnick and Hollingworth (1959) published an interesting account of the effect on rat erythrocytes of infection with *Bartonella muris*. They studied the life-span of erythrocytes in affected animals by the ^{51}Cr technique and concluded that the cells were irreversibly damaged. The Bartonellæ did not appear to penetrate the cell membrane; they appeared rather to adhere to the surface of the cells and were thought to cause local damage at the site of adhesion.

Bacteria which form powerful exotoxins can certainly cause lethal damage to erythrocytes as the result of a direct effect of the toxin. The α toxin of *Cl. welchii*, a lecithinase, probably brings about hæmolysis *in vivo* in this way; it acts on the lipoprotein complexes at the cell surfaces and causes the liberation of highly potent lytic lysolecithins. The marked spherocytosis of *Cl. welchii* infection may be looked upon as evidence of cell damage and as a stage in the lytic process. This type of direct toxic mechanism probably operates also in other septicæmias due to hæmolytic organisms, *e.g.*, in staphylococcal and β-hæmolytic streptococcal septicæmias, and also possibly in pneumococcal infections (Shumway, 1958; Shumway and Pollock, 1965).

Another way in which bacterial infection may injure erythrocytes is by a bacterial product bringing about direct agglutination. A wide variety of bacteria have been shown to be capable of acting in this way *in vitro* (Neter, 1956), but whether this mechanism often operates and brings about hæmolysis *in vivo* in man is unknown. Indirect types of "bacterogenic agglutination" are perhaps more important (see below).

2. Indirect Bacterogenic Agglutination. It is well known that polysaccharides derived from many species of bacteria can be adsorbed to human and animal erythrocytes (Keogh, North and Warburton, 1948) and that erythrocytes so treated are agglutinated by antisera directed against the adsorbed polysaccharide (Boyden, 1953; Neter, 1956). Many of the bacteria with which this phenomenon can be demonstrated *in vitro* are pathogens for man. The problem is whether this process of adsorption of bacterial antigen(s), and subsequent interaction of antibody and antigen(s) at the erythrocyte surface, can occur *in vivo* and, if so, whether this can produce hæmolysis. Experimental and clinical studies have shown that in-vivo sensitization is possible but the occurrence of overt hæmolytic anæmia as a result does not seem to have been conclusively proved.

Ceppellini and de Gregorio (1953) experimented with rabbits and the Vi antigen of *Salm. typhi.* They showed, if rabbit erythrocytes were allowed to adsorb the antigen *in vitro* and then were reinjected into animals immunized against the Vi antigen, that the injected cells were rapidly removed from the circulation. They did not succeed, however, in sensitizing erythrocytes *in vivo* as the result of injecting large amounts of the antigen.

In man in-vivo sensitization does, however, appear to have been demonstrated in rare instances, as for instance in the two infants described by Young, Gillem and Akeroyd (1962), who were suffering from *E. coli* enteritis. The antiglobulin test was positive with each infant's erythrocytes, using anti-human-globulin sera, and also when their erythrocytes were exposed to anti-*E. coli* immune rabbit sera and then treated with an anti-rabbit-globulin serum. Presumably in these cases the positive antiglobulin reaction with the anti-human-globulin serum was detecting anti-*E. coli* antibodies developed against adsorbed *E. coli* polysaccharide(s). This type of mechanism could explain the positive antiglobulin tests recorded in cases of typhoid and occasionally in other infections (see p. 910). It should be stressed, however, that the infants studied by Young, Gillem and Akeroyd were *not* reported to be anæmic, and whether the hæmolytic anæmias associated with typhoid and Salmonella infections can be explained on the above basis is unproved, although it is certainly tempting to accept the mechanism as a possible explanation.

Further experimental work was reported by Shumway, Bokkenheuser, Pollock and Neter (1963). Using a variety of bacterial polysaccharide antigens, they found that they dissociated *in vivo* from erythrocytes relatively rapidly, *i.e.*, within 5 days, and also that the presence of antibody inhibited antigen adsorption by erythrocytes *in vitro.* They concluded that in-vivo adsorption must be a rare event but thought that this could conceivably happen if the erythrocytes were exposed to high concentrations of antigen during the course of circulating through areas actually involved in the infection.

3. T-Type Antigen Transformation; Polyagglutinability etc. Another well studied way in which bacterial products may indirectly bring about agglutination of erythrocytes is through the unmasking of T antigens. Sera from healthy human beings normally contain anti-T antibodies and these cause the agglutination of T-transformed erythrocytes. This is the Hübener-Thomsen-Friedenreich phenomenon (see Part II, p. 588). It appears that enzymes derived from many bacteria (and viruses) can bring about this type of transformation *in vitro* and thus cause the cells to be polyagglutinable. Infection *in vivo* has seemed, too, in isolated incidences, to have led to polyagglutinability by this mechanism. This remains a possible rare cause of hæmolysis in human infections (Dausset, Moullec and Bernard, 1959).

An interesting hæmolytic system has recently been described by Evans and his co-workers in young rabbits suffering from mucoid enteritis (Evans, Bingham, Hickey and Hassett, 1959; Evans, Bingham and Weiser, 1963a, b). In this type of infection it is suggested that a bacterial product (? enzyme) renders the erythrocytes polyagglutinable by normally occurring and potentially agglutinating and hæmolytic plasma factors. Overt hæmolytic anæmia is thought not to occur because of rapid depletion of the plasma factors. This mechanism seems to be analogous to the T-type transformation in man.

4. Hypertrophy of the Reticulo-Endothelial System: Splenomegaly and Splenic Hypersequestration. The possibility that the cellular responses of the infected subject, in particular the hypertrophy of the spleen, which occur as the normal consequence of infection, might lead to increased destruction of erythrocytes has often been speculated upon, particularly in relation to diseases such as malaria and kala-azar (see p. 920), and subacute bacterial endocarditis and typhoid, in all of which splenomegaly occurs. This is an attractive hypothesis although it has proved difficult to obtain satisfactory evidence in its favour. However, Ho and Kass (1958) concluded, using rabbits injected with bacterial endotoxin, that an unexplained activation of the RE-cell system was a likely explanation for the mild hæmolytic anæmia which resulted. Jandl, Jacob and Daland (1961), too, who studied five patients with miscellaneous infections and demonstrated varying degrees of spherocytosis, splenic enlargement, shortened erythrocyte survival and increased splenic sequestration, suggested that the splenic enlargement was the primary event and that the increased hæmolysis was secondary to it. It must be added, however, that the phenomena they observed could equally well all have been the consequences of erythrocyte damage brought about by one or more of the mechanisms outlined in the previous paragraphs.

More recent clinical and experimental work has, however, provided further evidence supporting the concept of the non-specific activation of the RE-cell system by infection. Thus Sherman and Friedell (1962) obtained a positive correlation between the size of human liver abscesses and the consequent splenomegaly (and anæmia) and similar results were obtained by Betts, Tanguay and Friedell (1964) in hamsters in which turpentine abscesses were produced. Wagner, Iio and Hornick (1963), too, in human volunteers given typhoid and in patients suffering from pneumococcal pneumonia, found that the clearance of ^{131}I-aggregated human albumin was increased as the result of the infections. They suggested that the hypertrophied RE-cell system of infections should be looked upon not only as capable of phagocytosing microorganisms but also as a non-specific scavenger of "debris" left behind in the body as the result of the infection. (Erythrocytes damaged by any of the mechanisms mentioned in the preceding paragraphs could be looked upon as debris in the above context.)

5. Auto-Immune Hæmolytic Anæmia. The relationship between auto-immune hæmolytic anæmia and infection with viruses has been discussed in detail in Chapter 9 (Part II, p. 525), and also on p. 585. There is no disputing the fact that genuine anti-erythrocyte-antibody hæmolytic anæmia has often developed following virus infections, but, although concurrent bacterial infection may exacerbate pre-existing auto-immune hæmolytic anæmias, it still remains uncertain whether bacterial infection by itself is capable of initiating an auto-immune hæmolytic anæmia. This appeared to be possible in a few cases in the author's series (see Table 14, Part II, p. 343), and more recently Bedarida, Bernasconi and Pollini (1961), in reporting the presence of chronic tonsillar streptococcal sepsis in four patients with auto-immune hæmolytic anæmia, again raised the question as to a causal relationship. In the present author's view the case has certainly not been proved.

It should be added that the presence of positive direct antiglobulin tests cannot on their own be accepted as evidence that anti-erythrocyte antibodies are being produced, for a reaction between immune antibody and adsorbed bacterial polysaccharide has to be excluded (see above).

Summary

The anæmia of infection has a complicated origin and the part, if any, played by increased hæmolysis seems likely to vary according to the infecting organism and probably, for as yet unknown reasons, with the patient himself. The possible mechanisms range from direct parasitism of the erythrocytes by the infecting organism to the effects of hæmolytic toxins, and from complicated interactions between the patient's own antibody-containing plasma and erythrocytes which have adsorbed bacterial polysaccharides, or have been "altered" by bacterial enzymes, to "non-specific" effects resulting from reticulo-endothelial cell hyperplasia and hypersplenism. The problem is to find out which mechanism or combination of mechanisms is the more important in each individual instance.

PROTOZOAL INFECTIONS AND HÆMOLYTIC ANÆMIA

Malaria

There is a large literature on the occurrence of excess hæmolysis in malaria (see Zuckerman, 1964). Although blackwater fever is notorious as a striking and dangerous (although nowadays a very rare) complication of malaria, minor degrees of increased hæmolysis probably regularly accompany malaria in man irrespective of whether his illness is acute or chronic (see de Vries, 1946). It is a well known accompaniment, too, of the malarial therapy of GPI or other manifestation of syphilis (Hilton and Owen, 1963). In recent years, too, a few accounts of more severe hæmolytic anæmias (but not characterized predominantly by intravascular hæmolysis) have appeared in the literature. The patients described, who were suffering from acute or chronic malaria, responded to steroid therapy in much the same way as do patients with "idiopathic" auto-immune hæmolytic anæmia (Cook, Briggs and Horns, 1956; Demirağ and Sözer, 1956). The direct antiglobulin test was positive in Demirağ and Sözer's two patients.

Experimentally, it has been shown that the erythrocyte life-span is considerably shortened in malarial infection and that this can occur before the onset of parasitæmia and clinical manifestations of the disease (Stohlman, Contacos and Kuvin, 1963). The serum-haptoglobin levels fall markedly (Blumberg *et al.*, 1963).

Blackwater Fever

This classic type of acute hæmolytic anæmia with hæmoglobinuria has been the subject of many studies in the past. It was recognized as a serious complication of malaria towards the end of the last century, but reference to its occurrence can be found in medical writings long before this (Blackie, 1944). Geographically, blackwater fever has occurred chiefly in tropical and subtropical regions of Africa, in India, Ceylon and the Far East, in central America and in Macedonia. Racially, nearly all the victims have been Europeans. At the present time the disorder seems virtually to have become extinct and only a few cases have been seen and reported in recent years (*e.g.*, by Browne (1954) and Ryan and Parsons (1961)). This is no doubt the consequence of the success in controlling malaria throughout the world. The majority of cases have occurred in the course of infection with *P. falciparum* (malignant tertian malaria), and in many cases, although not in all, the actual hæmolytic crisis seems to have been precipitated by the taking of quinine or other anti-malarial drug (see Zuckerman (1964) for references to the literature).

Clinically, an attack is usually ushered in by a rigor associated with high pyrexia. Prostration and vomiting are common accompaniments. The urine varies in colour from dark red almost to black, and in severe cases oliguria or even anuria may occur. On the day following the start of the attack the patient is usually jaundiced. The clinical features are thus of an acute intravascular hæmolysis associated with general prostration and pyrexia.

Hæmatological Changes. The early account of Christophers and Bentley (1909) is noteworthy. The changes in the blood picture are often dramatic. The erythrocyte count may fall to low levels within a few hours; counts as low as 1,000,000 per cu. mm. have been recorded (Blackie, 1944). Stained peripheral blood films may reveal the presence of malaria parasites but often they are absent. The morphology of the surviving erythrocytes is not strikingly abnormal. There may be slight spherocytosis at the height of the paroxysm (Fairley and Murgatroyd, 1940; Foy and Kondi, 1943–4). During recovery, a reticulocytosis develops dependent upon the severity of the anæmia, with a corresponding degree of polychromasia and punctate basophilia in the fixed and stained blood film. Normoblasts and myelocytes may be present in the peripheral blood in small numbers at the height of the hæmolysis and during the early recovery phase.

Oxyhæmoglobin and methæmalbumin are characteristically found in the plasma (Fairley, 1941) as well as a raised bilirubin concentration, the latter persisting well into the recovery phase. Erythrocyte osmotic fragility is usually normal but the sensitivity to lysis by lysolecithin may be increased (Foy and Kondi, 1943–4; Foy, 1948).

The urine characteristically contains variable amounts of oxyhæmoglobin, methæmoglobin, urobilinogen, albumin and casts.

Serology. The direct antiglobulin test was negative in the few cases in which the reaction was carried out (Foy, 1948). Similarly attempts to demonstrate abnormal antibodies in the serum seem to have been unsuccessful (see also under *Pathogenesis*).

Pathology. In fatal cases the *spleen* is always found to be enlarged and engorged with blood. The littoral cells and reticulum cells of the pulp are hyperplastic and prominent and there is usually evidence of erythrophagocytosis. Malarial pigment may also be present. The *liver* appears congested with blood and engorged with bile pigment. Areas of necrosis may be visible. Malarial pigment may be seen in Kupffer cells. The *kidneys* show a variable degree of lower-nephron nephrosis with pigment-cast formation.

Aetiology and Pathogenesis. The exact cause or causes of the anæmia of malaria, and of blackwater fever in particular, are not known with certainty. Zuckerman (1964), in a long and detailed review, stressed the fact that the severity of anæmia in malaria implies that non-parasitized as well as parasitized erythrocytes have a shortened life-span. She listed five possible hæmolytic mechanisms: (1) the hæmolytic action of anti-malarial drugs; (2) the possibility that soluble plasmodial antigen may coat erythrocytes and that interaction between antigen and the corresponding antibody may take place on the erythrocyte surface; (3) that heterophile antibodies developed against plasmodia may, because of shared antigens, react with erythrocytes; (4) that tissue lysins may be a factor in hæmolysis, and (5) that the host cells (in particular, the erythrocytes) may be altered by the parasites so as to become auto-antigenic.

Zuckerman also discussed the role of hypersplenism as an accessory mechanism and pointed out that although splenectomy might temporarily inhibit the anæmia of (experimental) malaria it exacerbated the parasitæmia; she concluded by coming down rather strongly in favour of the auto-immune hypothesis, although in the present author's opinion the evidence for this is not particularly strong.

There seems no doubt that treatment with an 8-aminoquinoline drug can provoke in a subject who has malaria and G-6-PD deficiency a hæmolytic crisis which could be mistaken for blackwater fever. On the other hand, blackwater fever existed long before the modern anti-malarial drugs were synthesized; moreover, Europeans have, both in India and Africa, been more subject to blackwater fever than the native population. The role of quinine in provoking an attack appears to be definite, even if the mechanism of hæmolysis is obscure. The sequence of events suggests a hypersensitivity reaction to drug or parasite (or killed parasite or products derived from it), and the erythrocytes perhaps get involved because of antigen-antibody reactions occurring in the blood stream. It is fairly easy to speculate; perhaps because of the virtual elimination of blackwater fever the truth will never be known.

The more chronic and less dramatic anæmias of malaria are probably more easily explainable by destruction of parasitized corpuscles and by hypersplenism and general reticulo-endothelial cell hyperplasia. Possibly in rather rare instances minor modifications in erythrocyte antigenicity, brought about perhaps by the presence of the parasites within the cells, coupled with an unusual proneness of the individual patient to develop antibodies, lead to an auto-immune type of hæmolytic anæmia. But this, too, is speculation.

Leishmaniasis (Kala-Azar)

Excess hæmolysis appears to be an important cause of anæmia in at least some patients suffering from kala-azar. Napier and Sharma (1933) in an early study concluded that there was considerable evidence in favour of increased blood destruction and suggested that this was probably brought about by "hyperactivity of the normal mechanism of erythrolysis." They pointed out that a marked proliferation of the RE-cell system was the characteristic pathological feature of generalized Leishmania infection. More recent papers stressing the role of hypersplenism in the pathogenesis of the anæmia include those of Burchenal, Bowers and Haedicke (1947), Rachmilewitz, Braun and de Vries (1947), Rachmilewitz, De Vries and Gurevitch (1952) and Zuckerman (1964). Martins, Alencar and Magalhâes (1965) reported on hæmatological data obtained from a large series of patients suffering from kala-azar in Brazil; they, too, concluded that increased hæmolysis plays an important role in the pathogenesis of the anæmia.

Relapsing Fever

Banwell and Kibukamusoke (1963) described a patient with relapsing fever who had conspicuous anæmia and jaundice and concluded that the anæmia was hæmolytic in origin.

Toxoplasmosis

This infection has been held to be responsible for acute or chronic hæmolytic anæmia in childhood (Hoppelar, Brison, Papillon and Chardac, 1960) or adult life (Kalderon, Kikkawa and Bernstein, 1964). Infection in infancy has been reported, too, to produce a blood picture simulating that of hæmolytic disease of the newborn (Zuelzer, 1944).

HÆMOLYTIC ANÆMIA ASSOCIATED WITH SARCOIDOSIS

Several instances of hæmolytic anæmia have been reported in patients suffering from, or who subsequently developed, sarcoidosis (Besnier-Boeck-Schaumann disease). The exact relationship between the two disease processes remains obscure but the reports appear to be sufficient in number to rule out coincidence.

Crane and Zetlin (1945) described a patient, aged 46, who underwent splenectomy for hæmolytic anæmia; she improved temporarily but relapsed 2 months later. There was marked spherocytosis and a plasma-globulin concentration of 4 g. per 100 ml. At necropsy the characteristic histological appearances of sarcoid were found in lymph nodes and bone marrow.

Stats, Rosenthal and Wasserman (1947) reported the history of a male Negro child who developed severe hæmolytic anæmia when 6 months old. When aged 7½ years, hæmolysis recurred in association with fever, enlargement of the liver and spleen, and spherocytosis. One year later iridocyclitis developed and a lymph-node biopsy showed sarcoid.

McCort, Wood, Hamilton and Ehrlich (1947) described the case of a man, aged 58, who was known to have enlarged mediastinal lymph nodes, the histological picture of which was typical of sarcoid. Two years later hæmolytic anæmia developed, which was not relieved by splenectomy. There was hyperglobulinæmia, and radiographs showed that the nodes had increased in size.

Bruschi and Howe (1950) reviewed the early literature on blood changes in sarcoidosis and described a further patient, a 45-year-old female, who developed severe hæmolytic anæmia. Osmotic fragility was normal. Splenectomy was followed by temporary improvement only. Sarcoid was demonstrated histologically in the spleen, in a splenic lymph node and in the liver.

These early reports suggested that the presence of sarcoidosis either brings out latent hæmolytic anæmia in some way, as in Stats, Rosenthal and Wasserman's case, or initiates hæmolysis by an auto-immune or some other mechanism. There have been a number of subsequent reports, some with positive and others with negative direct antiglobulin tests.

Schubothe (1952) described the occurrence of a chronic hæmolytic anæmia in a child aged 12 years who was known to have sacroid changes in the lungs. The direct antiglobulin test was positive. Remission followed treatment with cortisone.

Davis, Belber and Movitt's (1954) patient was a 34-year-old Negro who developed a severe hæmolytic anæmia; the antiglobulin test was negative. Splenectomy was carried out without much benefit but he did respond to cortisone. Sections of the spleen showed a massive infiltration by sarcoid lesions.

Lebacq, Tirzmalis, Mairiaux and Pluygers (1956) described a patient with splenomegaly who underwent splenectomy for hypersplenism (? Banti's disease). Sections, however, showed an enormous number of sarcoid nodules. Six months later severe hæmolytic anæmia developed and the direct antiglobulin test was strongly positive. The anæmia responded to ACTH therapy. An interesting further point is that this patient's sister had previously undergone splenectomy for splenomegaly, and sarcoid lesions had been found in her spleen, too. A further patient with a hæmolytic anæmia, a positive antiglobulin test and sarcoid-containing lymph nodes was described by Michon and his colleagues (1957). Other more recent case reports include those of Garcia, Garrido, Lorenzo and Guedes (1959), West (1959) and Cox and Donald (1964).

West (1959) referred to 1,000 splenectomies carried out at Columbus, Ohio, for cytopenic syndromes. Six patients had sarcoid lesions in their spleens; five of them had suffered from "ITP" and one had hæmolytic anæmia. West's own patient was a 32-year-old woman who gave a 6 months' history of jaundice and anæmia. A chest radiograph showed enlarged mediastinal lymph nodes and biopsy revealed sarcoid. Splenectomy was followed by some improvement in her severe hæmolytic anæmia. The direct antiglobulin test was negative.

Hæmolytic anæmia is clearly a rare complication of sarcoidosis. Its origin is mysterious and the above-quoted case reports suggest that more than one mechanism may operate. One factor may be an abnormal immune responsiveness: perhaps the high serum-globulin levels so often found in sarcoidosis are a reflection of this (Ricker and Clark, 1949). The often poor response to splenectomy suggests that hypersplenism can be no more than a part explanation.

HÆMOLYTIC ANÆMIA IN PREGNANCY

The effect of pregnancy on the course of a hereditary or chronic acquired hæmolytic anæmia is well-documented, and where there is a hæmoglobinopathy, in Hb-S disease and Hb-S/Hb-C disease in particular, it may constitute a dangerous hazard (see Part I, pp. 257 and 268). In hereditary spherocytosis and elliptocytosis, on the other hand, and in pyruvate-kinase deficiency, pregnancy does not seem usually to be a particularly serious event and the patients are able as a rule to maintain a reasonably good hæmoglobin level. In paroxysmal nocturnal hæmoglobinuria thrombotic and septic complications may be more than normally frequent. In all these conditions, however, there is a definite risk of folic-acid deficiency and it would be no doubt wise to administer small supplements of folic acid throughout the latter two trimesters of pregnancy. Thus, overt megaloblastic anæmia occurring in pregnancy was recorded in cases of hereditary spherocytosis by Davidson (1952) and Drury and Geoghegan (1957) and in an obscure case of non-spherocytic hæmolytic anæmia by Söderhjelm (1959). The precipitating factor in the hæmolytic crises occurring in four successive pregnancies in the case of hereditary spherocytosis described by Rimbach and Beickert (1955) may also have been temporary folic-acid deficiency.

Folic-acid deficiency may also complicate acute or chronic acquired hæmolytic anæmia during pregnancy, as, for example, in the case report of Bateman, Hutt and Norris (1959).

In all the above conditions, too, the chances of miscarriage are probably increased. Borglin and Hackl (1964), in a review of 34 published cases of various types of hæmolytic anæmia in pregnancy and 12 cases of their own, stressed the frequency of crises of hæmolysis; but they also reported that the abortion rate was 22% and that the perinatal infant mortality (including death from prematurity) was 9%.

Of special interest are the cases of hæmolytic anæmia which develop for the first time in pregnancy and, in particular, those in which excess hæmolysis has recurred in a subsequent pregnancy. Their occurrence raises the question as to whether pregnancy *per se* can provoke hæmolytic anæmia.

Incidence. Hæmolytic anæmia arising apparently for the first time in pregnancy is a distinctly rare event. Lillie, Gatenby and Moore (1954) described one such example in 4,314 pregnancies. Borglin and Hackl (1964) referred to 10 cases. More than one pathogenetic mechanism may operate: some cases appear to be auto-immune in origin, others not. (The association between eclampsia or pre-eclampsia and hæmolytic anæmia during pregnancy or after delivery has already been discussed (p. 876) and will not be dealt with again in this Chapter.)

The early reports suffer inevitably from incomplete investigation. Lescher's (1942) series of eight cases of acute hæmolytic anæmia ("Lederer type") comes into this category. This mostly occurred *post partum* and in two patients at least sepsis appears to have been a major factor in causation.

Hæmolytic Anæmia Occurring in more than one Pregnancy. In 1941, the present author, in collaboration with the late Dr. E. ff. Creed and Professor P. L. Mollison, studied a patient who suddenly developed a severe hæmolytic anæmia when 30 weeks' pregnant. There was no spherocytosis but erythrocyte survival studies (published by Mollison, 1947) showed that normal transfused cells were completely eliminated (on two occasions) within 20 days. Cæsarean section was eventually carried out and the blood picture was found to be normal 4 months later. The particular interest in this case is that this patient developed what appeared to be a similar episode in a subsequent pregnancy (in the late 1940's). On this occasion the direct antiglobulin test carried out on a blood sample kindly provided by Professor W. M. Davidson was found to be negative. The exact status of this patient in the intervening years is not known.

A superficially similar case was recorded by Bromberg, Toaff and Ehrenfeld (1948). This patient developed severe hæmolytic crises in her 8th and 9th pregnancies; she was, however, known to remain fairly severely anæmic and to have a raised reticulocyte count in the intervening period. Another somewhat similar case was published by Zachariae (1953). This patient suffered from hæmolytic anæmia during her 3rd, 4th and 5th pregnancies, as well as at least one further episode of hæmolysis between the 4th and 5th pregnancies.

More important are the observations of Craig and Turner (1955). Their patient's history resembles that of Creed's case (see above). She developed severe hæmolytic anæmia during the latter half of two successive pregnancies but in each instance recovered from the anæmia after delivery. Spherocytes were absent from the blood film, osmotic fragility was normal and all tests for auto-antibodies were negative. Transfusion produced only transient rises in hæmoglobin and this was taken to indicate that the hæmolytic mechanism was not an intrinsic one.

Other Recent Case Reports. More recently, Batemen, Hutt and Norris (1959) and Jankelowitz, Eckerling and Joshua (1960) have published accounts of single patients suffering from severe hæmolytic anæmia, of obscure origin, which developed in pregnancy. Both patients appeared to benefit from treatment with adrenocorticosteroids (although serological tests for auto-antibodies were negative) and in both instances a lesser degree of anæmia persisted for some months *post partum.*

As already mentioned, there are a few descriptions in the literature of apparent auto-immune hæmolytic anæmia occurring in pregnancy, *e.g.*, the case reports of Wagner and Maresch (1955), Wirtheimer (1957), and Burt and Prichard (1957). The anæmia of Burt and Prichard's patient antedated her pregnancy, and it is interesting to note that the infant developed severe hæmolytic jaundice after birth and needed two exchange transfusions, although its direct antiglobulin test was negative.

The mother underwent splenectomy during pregnancy, and she apparently recovered from her anæmia after delivery.

Pathogenesis

From the above account it is obvious that little is known of the ætiology and pathogenesis of the acute hæmolytic anæmias which occasionally develop during pregnancy and of the part that pregnancy plays in their causation. However, it is clear that, although pregnancy can have a serious effect on the course of some pre-existing hæmolytic anæmias, this is not always the case, and it is also clear that folic-acid deficiency—probably due to increased demands of the mother and fœtus—may lead to exacerbation of the anæmia and megaloblastic change in the marrow. Beyond this nothing is certain. But the possibility that pregnancy *per se* can in some way initiate severe hæmolysis cannot be dismissed. How and why this happens are at the moment quite obscure.

It is known, or at least supposed, that the endocrine changes associated with menstruation may affect the rate of hæmolysis of erythrocytes (Ashby, 1921; Callender, Powell and Witts, 1947; Berlin, 1951). Berlin found in five cases that there appeared to be an increased elimination of erythrocytes from the circulation during the 6–10 days before normal menstruation, but whether an exacerbation of the causal mechanism (apparently operating in the progesterone phase of the menstrual cycle) is relevant to grossly excess hæmolysis occurring in pregnancy is unknown. It should be added that minor increases in osmotic fragility have been reported in both anæmic and non-anæmic pregnant women (Elliott, 1944), but the significance of this finding, too, is uncertain.

"HYPERSPLENISM"

The role that the spleen plays in the various types of hereditary and acquired hæmolytic anæmia has already been discussed in considerable detail in earlier pages of this book. The problem now to be discussed is whether hyperplasia or other pathological process taking place within the spleen can by itself lead to increased blood destruction. In fact, as a rather rare occurrence, cases of hæmolytic anæmia do occur whose origin seems to lie solely (or largely) in increased splenic hæmolysis, *i.e.*, in "hypersplenism". In most instances these patients suffer in addition from varying degrees of leucopenia and thrombocytopenia.

Panhypersplenism (Hypersplenic Pancytopenia)

The early literature is inextricably tangled and confused, for not only has the word "hypersplenism" been used to describe the blood picture (anæmia, leucopenia and thrombocytopenia) so often found in association with massive enlargement of the spleen, of very varied causation, but it has also been used to describe a supposed "primary" splenic disorder ("splenic panhematopenia" (Doan and Wright, 1946); "primary hypersplenism" (Doan, 1949)). Doan (1949) also included under the title "primary hypersplenism" hereditary spherocytosis and even idiopathic thrombocytopenic purpura.

Crosby (1962a), too, in an excellent review widened the concept still further by stating that: "when a person is hematologically better off without his spleen he has hypersplenism." But it is interesting to note that Crosby, while giving detailed descriptions of hypersplenic anæmia, thrombocytopenia and leucopenia as occurring in a variety of blood disorders and splenomegalies, does not seem to mention "primary hypersplenism," presumably because he did not feel there was sufficient evidence for the existence of such a condition.

It is the present author's experience, however, that patients do present themselves from time to time with striking splenomegaly, of unknown origin, associated with varying degrees of anæmia (often indisputably hæmolytic, in part at least), leucopenia and thrombocytopenia, and that clinical and as a rule long-continued hæmatological cure follow if splenectomy is carried out. The histological findings in the spleens of these cases vary, but some at least show hyperplasia, particularly of lymphoid tissue, rather than neoplasia or other definable disease (Brain, Dacie, Galton, Harrison and Lewis, 1966). If there is such a disease as "primary hypersplenism," some at least of these patients certainly come close to suffering from it.

Literature on hypersplenism. This is surprisingly scanty. Crosby's (1962a) review has already been referred to. The following papers include descriptions of patients whose illness is described under a variety of titles but who may in fact have been suffering from "primary" or "idiopathic" hypersplenism. Wiseman and Doan (1942) described cases of *primary splenic neutropenia* (with evidence for probable hæmolysis in four out of five cases); Dausset, Paraf and Caroli (1952) a case of *anémie hémolytique hypersplénique*; McFadzean, Todd and Tsang (1958) cases of *cryptogenetic splenomegaly* in Hong Kong, and Gevirtz, Nathan and Berlin (1962) a case of *primary hypersplenism.*

Data concerning the patients the author and his colleagues have personally studied are summarized in Table 68. Details of three of these patients (Cases 34–36) selected to illustrate individual variation within the syndrome of "primary hypersplenism" are given below.

Case Report: "Primary Hypersplenism"; Incomplete Response to Splenectomy

Case 34. The patient, A. D., was an unmarried woman aged 64. She had been well until 1954 when she developed a sinus infection which persisted and led to chronic nasal obstruction. In 1954, too, she started to experience palpitations and shortness of breath and was noticed to be pale and yellowish in colour. These symptoms and signs increased over the following 2 years and towards the end of this time swelling of the ankles had been noticed. Four months prior to admission she had been treated with intramuscular vitamin B_{12} as a case of pernicious anæmia, but without response; she had, however, developed an abscess at an injection site. She was admitted into Hammersmith Hospital on the 12th September 1956.

Physical Examination. She was pale and slightly jaundiced and there was minimal œdema of the ankles. The liver was palpable 2 cm. below the right costal margin and the spleen was palpable 5 cm. below the left costal margin; the superficial lymph nodes did not appear to be enlarged.

Laboratory Findings. On admission, the hæmoglobin was 7·5 g. per 100 ml., PCV 25%, MCV 119 cu.μ and reticulocyte count 20%. The total leucocyte count varied between 250 and 1,800 per cu. mm. with 1–12% neutrophils; the platelet count ranged between 26,000 and 116,000 per cu. mm.

Stained films showed slight anisocytosis and macrocytosis, and a moderate degree of polychromasia (Fig. 233). Osmotic fragility was normal: lysis commenced in 0·5% NaCl and the MCF was 0·43% NaCl. The direct antiglobulin test was negative.

The serum bilirubin was 1·0 mg. per 100 ml. and serum proteins 7·7 g. per 100 ml., albumin 2·7 g. and globulins 5·0 g. Electrophoresis showed a broad increase in gamma globulins; thymol turbidity was 6 units and zinc sulphate precipitation 12 units.

Bone-marrow biopsy revealed a richly cellular marrow in which erythropoiesis, leucopoiesis and thrombopoiesis were unusually active; many metamyelocytes were present but only a very few segmented neutrophils. The findings were interpreted as showing active hæmopoiesis in response to an increased rate of peripheral destruction and to be compatible with a diagnosis of hypersplenism.

Treatment and Progress (Fig. 235). A tentative diagnosis of hypersplenism, ? "primary" type, was made. The cause of the rise in gamma globulins was obscure, but it seemed possible that this was a response to long-continued chronic sepsis. Splenectomy was undertaken on the 19th October 1956 and a spleen weighing 1,470 g. was successfully removed.

Splenectomy was followed by a good clinical response. The patient lost her jaundice, the hæmoglobin rose and the reticulocyte count fell to normal. The leucocyte count did not, however, rise as much as was expected: the maximum recorded count was 3,600 per cu. mm. and the maximal neutrophil count 1,370 per cu. mm. (Fig. 235). The platelet count, however, rose substantially, reaching a maximum of 430,000 per cu. mm.

The patient was discharged from hospital on the 21st November 1956, just over 1 month after splenectomy; the hæmoglobin was then 12·3 g. per 100 ml., reticulocytes 0·6%, leucocyte count 2,400 per cu. mm. and platelet count 350,000 per cu. mm. The serum bilirubin was 0·3 mg. per 100 ml.

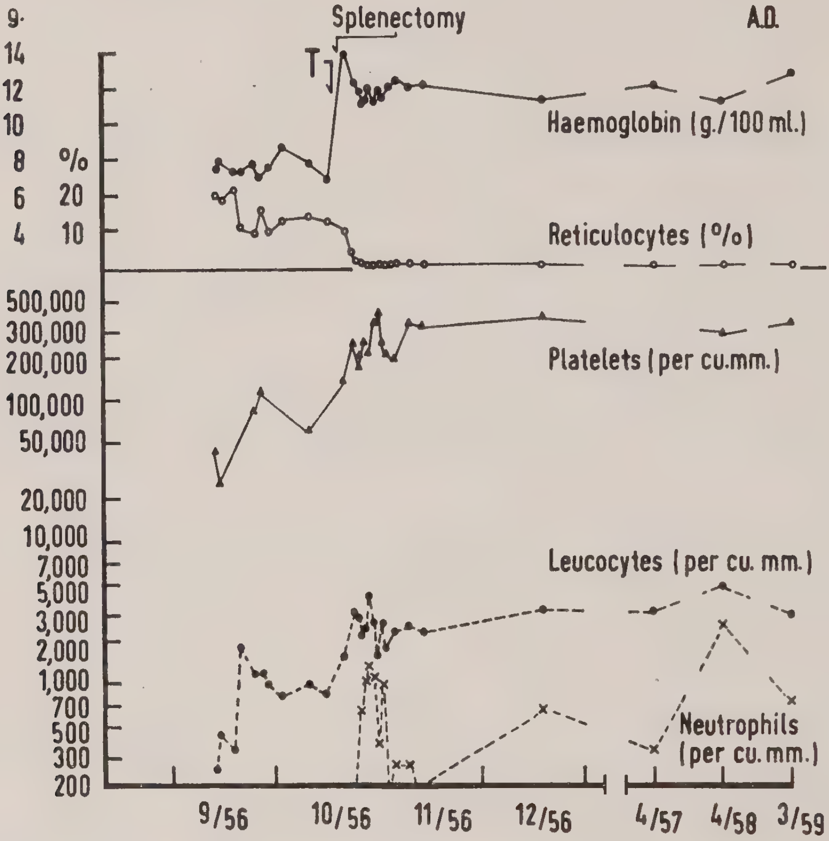

FIG. 235. Hæmatological findings and the effect of splenectomy in a patient who suffered from "primary hypersplenism" (Case 34). T denotes blood transfusion.

Histology of Spleen. The lymphoid follicles were enlarged and some had germinal centres; the reticulin pattern and the orientation of lymphocytes in the follicles was normal (Prof. C. V. Harrison). The red pulp was cellular and contained a moderate amount of blood; erythrophagocytosis was difficult to see but large amounts of iron were present in phagocytic cells. Scattered plasma cells could be identified but neutrophils were inconspicuous.

Follow-up. The patient has been seen from time to time by Dr. A. H. Banton who has kindly supplied the following information. She kept well after splenectomy and when last seen in March 1959 she had gained 3 stone (19 kg.) in weight. Her hæmoglobin had been maintained between 10·7 and 12·9 g. per 100 ml. and the reticulocyte count at

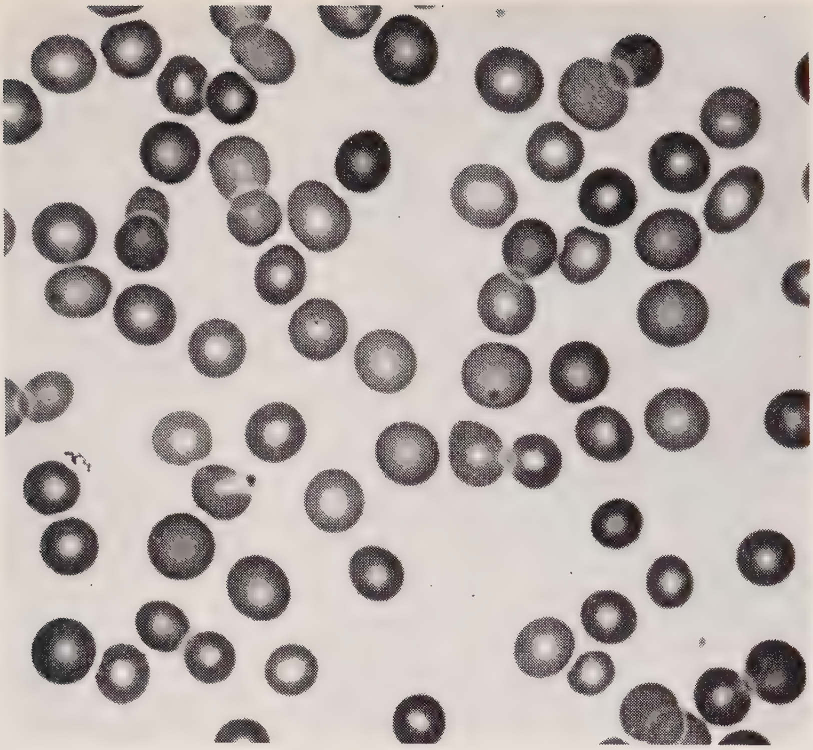

FIG. 233. Photomicrograph of a blood film of a patient suffering from "primary hypersplenism" (Case 34). There is slight anisocytosis and macrocytosis. ×700.

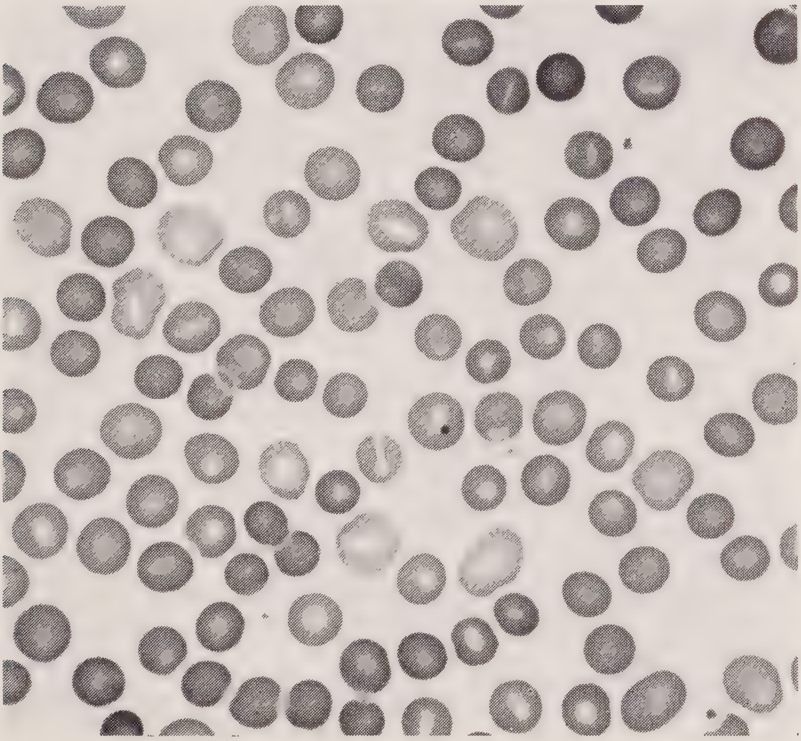

FIG. 234. Photomicrograph of a blood film of a patient suffering from "primary hypersplenism," who gave a positive direct antiglobulin test (Case 35). There is considerable anisocytosis and microspherocytosis, and a little macrocytosis. ×700.

less than 1%. The total leucocyte count had ranged between 3,100 and 5,000 per cu. mm., but the neutrophil count was markedly low in three out of four counts (340–740 per cu. mm.); on the fourth occasion it was 2,750 per cu. mm., near the lower limit of normal. The platelet count remained at a high normal level (300,000–400,000 per cu. mm.).

Summary. This patient showed the clinical and hæmatological features of hypersplenism. Splenectomy was followed by a good clinical result although neutropenia persisted. Sections of the spleen showed enlargement of the lymphoid follicles but no evidence of a lymphoma.

Aetiology and Pathogenesis of Hypersplenism

As so ably summed up by Crosby (1962a), the to-and-fro controversy between the splenic blood-cell destruction theories of Doan and the marrow-inhibition theories of Dameshek, which were hotly contested in the late 1940's and early 1950's, has now largely burnt itself out, and Dameshek's smiling concession, "Well, it looks as though Charley Doan is right," is seldom contested at the present time. There is in fact good evidence in man and in experimental animals in favour of splenic destruction, and not a great deal of solid evidence in favour of the marrow-inhibition hypothesis (Crosby, 1962b).

Experimental Evidence

A fruitful line of experimental work has stemmed from the observations of Hueper and his colleagues on the effects on the body tissues of the presence of macromolecular substances such as polyvinyl alcohol and methyl cellulose (Hueper, Landsberg and Eskridge, 1940; Hueper, 1942). Subsequently, the changes in the blood picture which follow the injection into animals of methyl cellulose have been extensively studied (Palmer *et al.*, 1953; Giblett *et al.*, 1956; Teoh, 1961; Rowley, Fitch and Bye, 1962). In these animals the hæmolytic anæmia which develops appears to be due to a great enlargement of the pulp compartment of the spleen with consequent sequestration of erythrocytes and increased opportunities for phagocytosis by hyperplastic reticulum cells.

Hyperplasia of the spleen, if accompanied by an enlargement of the pulp spaces and hyperplasia of phagocytic cells, could, on the basis of the above-quoted animal work, be the explanation for the increased hæmolysis in human hypersplenism. But whether it is necessary to postulate some increase above normal of the phagocytic activity of the reticulum cells of the spleen over and above an increase in numbers remains uncertain.

Stefanini, Xefteris, Moschides and Blumenthal (1961) discussed the lytic (and inhibitory) factors which can be demonstrated in tissue extracts and postulated that in pathological states, and in splenic tissue, an increase in tissue lytic activity might play a role in bringing about erythrocyte destruction, particularly in hæmolytic states in which there was no evidence for an immunological mechanism.

Role of Auto-Antibodies. A few cases have been observed in which massive splenomegaly and leucopenia and thrombopenia have been accompanied by the presence of a positive direct antiglobulin test or other abnormal serological findings (Sokal, 1957; Meuderissen and van Wageningen, 1959; Brain *et al.*, 1966). In these patients it cannot be denied that the development of auto-antibodies against erythrocytes may also have played a part in bringing about excessive hæmolysis. These patients certainly present a problem of classification for those who insist on separating their patients into rigid compartments—the present author does not. It can be stressed that in the majority of patients who might be considered to be suffering from "primary hypersplenism" tests for auto-antibodies against erythrocytes (or leucocytes or platelets) give consistently negative results. However, the present author has investigated two patients whose erythrocytes gave positive antiglobulin tests, but who otherwise appeared to be suffering from "primary hypersplenism." The case history of one of these patients is described below (Case 35).

Case Report: Hypersplenism Associated with a Positive Antiglobulin Test or (*?*) *Auto-Immune Hæmolytic Anæmia Associated with Hypersplenism*

Case 35. The patient, E. M., was a married woman aged 61. She gave a history of an attack of biliary colic in February 1953 for which cholecystotomy was performed. Previously she had been well. Early in 1954 she suffered from several episodes of nausea and vomiting and this led to her admission to Hammersmith Hospital in April 1954.

Physical Examination. She was found to be pale and slightly jaundiced and appeared to have lost weight. The liver edge was just palpable but the spleen was grossly enlarged to just below the umbilicus; it reached the mid-line of the abdomen. The superficial lymph nodes were not enlarged.

Laboratory Investigations. The hæmoglobin on admission was 7·7 g. per 100 ml. and this subsequently fell to a minimum of 6·1 g. per 100 ml. The reticulocyte count ranged between 5·4% and 19%. The total leucocyte count varied from 1,100 to 2,100 with from 50–64% neutrophils. The platelet count ranged between 102,000 and 160,000 per cu. mm.

Stained films showed a moderate degree of anisocytosis, and microspherocytosis but a few macrocytes were present also (Fig. 234). Osmotic fragility was slightly increased; the MCF was 0·47% NaCl and lysis began at 0·525% NaCl. The serum bilirubin was 4·9 mg. per 100 ml. and the blood urea 21 mg. per 100 ml. The total serum proteins were 6·0 g. per 100 ml., albumin 3·3 g. and globulins 2·7 g. The urine contained an excess of urobilinogen, but was otherwise normal.

Serological Findings. The direct antiglobulin test was positive and the reaction appeared to be a non-γ one. The cold-agglutinin titre was 16 (at 4° C.), with a trace of agglutination at 20° C. Enzyme-treated cells were agglutinated but not lysed at 37° C. to a titre of 4. The indirect antiglobulin test was negative at 37° C. An eluate was prepared from the patient's erythrocytes but no antibodies could be detected in it.

Further Progress (Fig. 236). Because of the large size of the spleen and the moderately severe leucopenia splenectomy was recommended; it was carried out on the 12th May 1954. A small contracted chronically inflamed gall bladder containing a cholesterol stone was also removed.

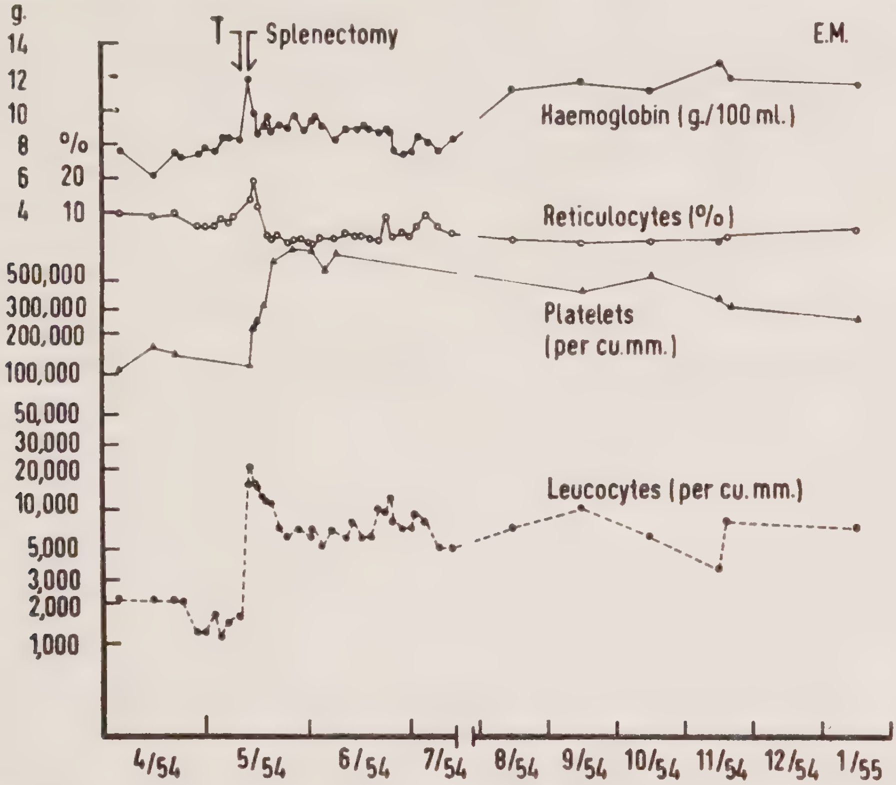

FIG. 236. Hæmatological findings and effect of splenectomy in a patient suffering from (?) "primary hypersplenism" (Case 35). The direct antiglobulin test was positive. T denotes blood transfusion.

The response to the operation was satisfactory, although a subphrenic abscess developed as a complication. This, however, responded to drainage and Aureomycin therapy. She lost her jaundice and the hæmoglobin rose to 11–12 g. per 100 ml. although the reticulocyte count did not entirely return to normal. The total leucocyte count rose post-operatively to 20,000 per cu. mm. and eventually stabilized within the normal range. The platelet count rose to a maximum of 1,300,000 per cu. mm. and then gradually fell. When last seen in January 1955, 8 months after splenectomy, she felt well and had a hæmoglobin of 11·4 g. per 100 ml., with 5·2% reticulocytes; the leucocyte count was 7,000 per cu. mm. and the platelet count 512,000 per cu. mm. The antiglobulin test was still positive.

Histology of Spleen. The spleen weighed 1,475 g. and was congested with blood; the lymphoid follicles were macroscopically enlarged. Sections showed that the architecture of the spleen pulp was normal; the red pulp was engorged with blood and the sinus littoral cells were enlarged and often cuboidal in appearance. There was some siderosis. The lymphoid follicles varied in size but on the whole they were considerably enlarged and in some areas were almost confluent; their structure and cytology was, however, normal (Prof. C. V. Harrison). Several abdominal lymph nodes, removed at the time of splenectomy, showed a normal structure; erythrophagocytosis was, however, a striking feature. A biopsy specimen of the liver showed a few lymphocytes in the portal tracts but was otherwise normal.

Summary. The patient presented with clinical and hæmatological signs suggestive of "primary hypersplenism", except for one unusual feature—a positive antiglobulin test. Splenectomy was followed by a good remission although slight anæmia and reticulocytosis, and a positive antiglobulin reaction, persisted.

Histology of the Spleen in Hypersplenism

Leffler (1952), who examined 83 spleens derived from patients considered to suffer from "hypersplenism", thought that he had detected a distinct abnormality which was seldom present in control spleens, namely a prominent marginal zone (a well delineated pale rim) to the Malpighian corpuscles. Unfortunately, most of his patients suffered from hereditary spherocytosis or idiopathic thrombocytopenic purpura and only nine from "idiopathic splenic hematopenia." The significance of Leffler's observations is thus not clear. In the cases investigated by the present author and his colleagues the major abnormality was a general hyperplasia of the spleen, with the lymphoid follicles in particular being strikingly hyperplastic although somewhat variable in cellular content from case to case. The structure of the spleen as revealed by its reticulin pattern was intact, but in most cases the reticulum cells of the red pulp and the littoral cells of the sinuses appeared to be hyperplastic. The red pulp contained a variable amount of blood in which neutrophils were often conspicuous and was in some instances infiltrated quite markedly by lymphocytes. Erythrophagocytosis was not easily seen but most of the spleens contained a moderate to a great excess of iron.

The lymphoid hyperplasia appeared to be benign in type rather than malignant in most instances, *i.e.*, a positive diagnosis of a lymphoma did not appear to be justified (Brain *et al.*, 1966). This interpretation of the findings fits in well with the generally good clinical benefit of splenectomy (see Table 68). It is, however, admittedly sometimes difficult to distinguish with certainty between benign and malignant changes on histological grounds, and

Table 68

Hæmatological, Histological and Clinical Findings in Six Patients Splenectomized for "Primary Hypersplenism"

Case	Sex Age	Minimum hæmoglobin (g. per 100 ml.)	Maximum reticulocyte count (%)	Minimum leucocyte count (per cu. mm.)	Minimum neutrophil count (per cu. mm.)	Minimum platelet count (per cu. mm.)	Erythrocyte life-span ^{51}Cr T½	Weight of spleen (g.)	Summary of histology of spleen	Present status
A.D. Case 34)	F.64	6·9	22	250	3	26,000	8	1,470	Lymphoid follicles enlarged; many show germinal centres. Red pulp cellular; large excess of iron	Well 2½ years after splenectomy; moderate neutropenia has persisted
E.M. (Case 35)	F.61	6·1	19	1,100	800	102,000	...	1,475	Lymphoid follicles enlarged and almost confluent. Red pulp engorged; gross excess of iron	Well 8 months after splenectomy; mild hæmolytic anæmia has persisted
E.T.	F.48	6.0	7·1	500	30	69,000	25	1,940	Lymphoid follicles enlarged. Red pulp cellular; much iron; neutrophils conspicuous	Well 7 years after splenectomy; normal blood count
E.P.	F.57	7·5	2·0	800	380	59,000	28	4,000	Gross hyperplasia of lymphoid follicles. Red pulp cellular; neutrophils conspicuous	Well 3½ years after splenectomy; normal blood count
A.H. (Case 36)	F.57	10·6	3·2	3,700	1,080	82,000	28	2,000	Lymphoid follicles greatly enlarged, almost confluent. Neutrophils present in scanty red pulp	Well 9 months after splenectomy; normal blood count
V.W.	F.61	8·0	7·6	2,200	1,050	86,000	13	575	Lymphoid follicles enlarged; contain excess histiocytes and blasts. Red pulp cellular and congested; neutrophils conspicuous	Normal blood picture 3 months after splenectomy

it is thought that all grades of lymphoid-cell proliferation from clearly benign to overtly malignant probably occur, with a corresponding range of clinical presentations and prognosis.

Hypersplenism Associated with Hypogammaglobulinæmia

A number of adult patients have by now been described in whom moderate or severe grades of hypogammaglobulinæmia have developed apparently as an acquired phenomenon. These patients usually give a history of having suffered from repeated infections and while some have an underlying malignant lymphoproliferative disease (Shaw *et al.*, 1960), this does not seem to be true of all. Splenomegaly is commonly present in patients belonging to either group and in some patients at least the blood picture shows the changes of hypersplenism. Thus, signs suggestive of excessive hæmolysis (as well as leucopenia and thrombocytopenia) were recorded in the case histories reported by Prasad and Koza (1954), Rohn, Behnke and Bond (1955), Prasad, Reiner and Watson (1957) and Thompson and Johnson (1962). In the two patients of Prasad, Reiner and Watson splenectomy led to a considerable improvement in the blood picture.

It has been suggested that, in some patients at least, the hæmolysis is the consequence of splenic hypertrophy and reticulo-endothelial-cell hyperplasia occurring as part of the response to repeated infection (Prasad, Reiner and Watson, 1957; Thompson and Johnson, 1962).

The author has studied one patient with the hypersplenism-hypogammaglobulinæmia syndrome: in this instance the basis of the splenic enlargement was lymphoid hyperplasia which was considered to be benign. A short account of the relevant clinical and laboratory findings are given below (Case 36).

Case Report: Hypersplenism Associated with Hypogammaglobulinæmia

Case 36. A married woman, A. H., aged 57, gave a history of good health until May 1961 when she developed an itchy skin rash of unknown origin which persisted for 5 days or so; two further similar episodes were experienced in the next 2 years. In May 1963 she sustained a minor injury to a knee and carbon tetrachloride was applied to it in error. Five days later jaundice was noticed and this led to a medical examination at which an enlarged spleen was found. She also complained of undue fatigue, pale stools and dark urine. In November 1963 splenectomy was contemplated, and at this time the direct antiglobulin test was found to be positive. This led to the patient being referred to Hammersmith Hospital for further investigation.

Physical Examination. The liver was palpable 8 cm. below the right costal margin and the spleen was enlarged to 10 cm. below the left costal margin. The superficial lymph nodes were not enlarged. There was a trace of sacral œdema, but otherwise there were no other noteworthy abnormal physical signs.

Laboratory Findings. There was slight anæmia: the hæmoglobin ranged between 11·5 and 13·1 g. per 100 ml., with 0·4–2·8% reticulocytes; the leucocyte count varied between 3,700 per cu. mm. and 7,000 per cu. mm., with 40–63% neutrophils (1,720–4,620 per cu. mm.) and the platelet count varied between 102,000 and 180,000 per cu. mm. Stained films showed slight anisocytosis and ovalocytosis (Fig. 237), and no abnormal leucocytes were recognized.

Serum proteins: albumin 4·3 g. per 100 ml., globulins 1·9 g. per 100 ml. Analysis of the γ globulins showed hypogammaglobulinæmia: γG 160 mg. per 100 ml., γA 40 mg. per 100 ml., and γM 80 mg. per 100 ml. (Dr. John Hobbs). The serum bilirubin was 0·4 mg. per 100 ml., alkaline phosphatase 7 K.A. units, thymol turbidity 1 unit and zinc sulphate precipitation 1 unit. The serum transaminases, GOT and GPT, were not increased; the blood urea was 43 mg. per 100 ml. Bromsulphthalein retention was $<5\%$ and the prothrombin time was normal (13 sec.).

Liver biopsy showed some excess of lymphocytes and an occasional plasma cell in the portal tracts but not extending outside these areas; and there were no stigmata suggestive of hepatic parenchyma-cell disease.

Serology. The direct antiglobulin test was positive (+ +) using a wide-spectrum antiglobulin serum and an anti-non-γ-globulin serum, but it was negative with an anti-γG-globulin serum. The cold agglutinin titre was 4 at 4° C. and 1 at 20° C. Papainized cells were agglutinated to a titre of 4 at 20° C. and hæmolysed (and agglutinated) to a titre of 64 at 37° C. The indirect antiglobulin test was positive at 20° C. and 37° C., but only if the serum was acidified to pH 6·5–7·0; on one occasion weak hæmolysis of normal erythrocytes was detected at 37° C. at pH 6·5–7·0. The above reactions were generally stronger with stored cells than with fresh cells.

The abnormal antibody appeared to be "non-specific" when tested against a panel of erythrocytes of known genotypes. Serum complement activity was reduced (19 units) and the W.R. and T.P.I. test were negative. Tests for anti-nuclear factor and for thyroid and gastric auto-antibodies were negative.

Further Studies. A tentative diagnosis of hypersplenism with hypogammaglobulinæmia was made and the survival of the patient's own erythrocytes was studied by the ^{51}Cr method: the result was normal and no excess accumulation of radioactivity in the spleen was demonstrated. The question of splenectomy was considered but a decision was postponed as the patient appeared relatively well and the blood picture was not unsatisfactory.

Readmission for Splenectomy. The patient kept well for the next 18 months or so but then it appeared clear that the spleen was increasing in size. The patient was, therefore, readmitted to Hammersmith Hospital in June 1965. On examination the spleen was found to be palpable at the level of the umbilicus, and there was also tenderness over the gall bladder.

The blood picture, too, had deteriorated slightly. There was now a moderate pancytopenia: the hæmoglobin was 10·6 g. per 100 ml., with 1·0% reticulocytes, leucocytes 4,000 per cu. mm., with 27% neutrophils (1,080 per cu. mm.), and platelets 82,000 per cu. mm. The serum bilirubin was 0·6 mg. per 100 ml. The serological findings were unchanged, except that a weak direct antiglobulin reaction was now obtained using an anti-γG-globulin serum, in addition to a strong reaction using anti-non-γ-globulin sera. The hypogammaglobulinæmia and the hæmolytic antibody acting on enzyme-treated cells were still present.

Splenectomy was successfully carried out on the 22nd June 1965 by Mr. R. Franklin and a single large gallstone was removed at the same time. Recovery was uneventful and the patient was able to leave hospital 13 days after the operation. The hæmoglobin was then 12·7 g. per 100 ml., with 0·8% reticulocytes, leucocytes 10,000 per cu. mm., with 47% neutrophils (4,700 per cu. mm.) and platelets 248,000 per cu. mm. At the time of writing (about 1 year after splenectomy) the patient reports that she is feeling well; the blood count is normal.

The *spleen* weighed just over 2,000 g. Many whitish nodules 1–3 mm. in diameter, and easily visible to the naked eye, could be seen on its cut surface; these were thought to be enlarged lymphoid follicles. Several lymph nodes 1–3 cm. in diameter were present at the hilum of the spleen; on section these were seen to be uniformly pale pink in colour.

Histological examination of the spleen and lymph nodes showed hyperplasia of lymphoid tissue but no convincing evidence of neoplasia (Prof. C. V. Harrison). The lymphoid follicles in the spleen were large and sometimes confluent. The red pulp was relatively reduced in amount; the reticulum cells within it appeared not to be hyperplastic and there was a scattered infiltration by neutrophils. Little iron was demonstrable. The appearances were similar to those found in other cases of hypersplenism (Table 68).

Summary. Splenomegaly, in a middle-aged woman, was associated with moderate pancytopenia, hypogammaglobulinæmia, a positive direct antiglobulin test and antibody free in the serum which hæmolysed enzyme-treated erythrocytes. In the absence of evidence for any underlying disease a tentative diagnosis of "primary hypersplenism" was made. Splenectomy was followed by a good clinical and hæmatological response. Histological examination of the spleen and abdominal lymph nodes showed lymphoid hyperplasia but no overt malignant change.

Secondary Hypersplenism

The concept underlying the term "secondary hypersplenism" is that enlargement of the spleen as a consequence of disease *not* confined to the spleen may also lead to anæmia (of hæmolytic type) and leucopenia and thrombocytopenia, and it seems likely that here, too, the excess hæmolysis is brought about by enlargement of the splenic pulp compartment and hyperplasia of phagocytic cells. Thus hypersplenism probably contributes, for instance, to the hæmolytic anæmia in many types of infection (p. 915), myelo-

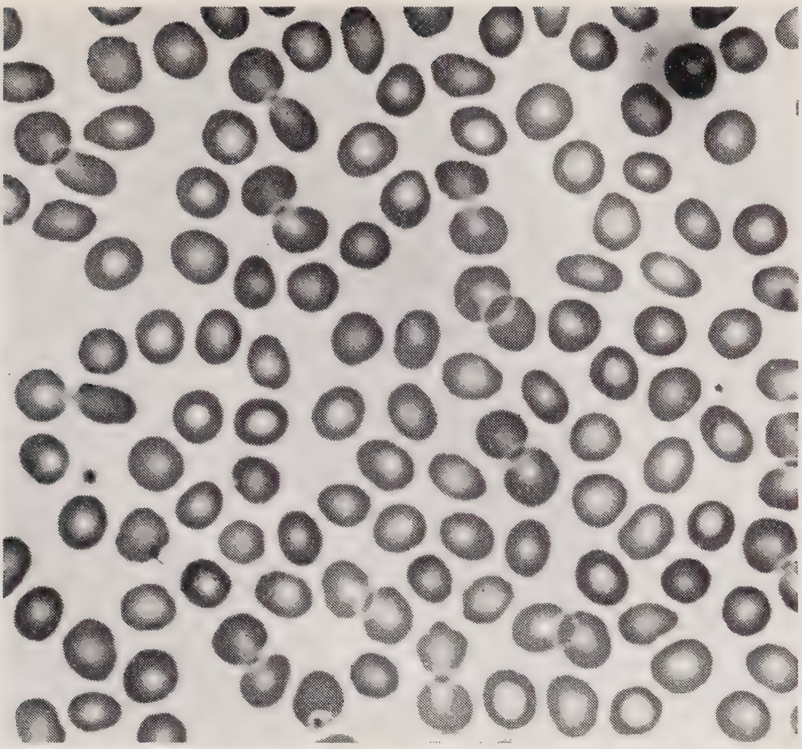

FIG. 237. Photomicrograph of a blood film of a patient who suffered from "primary hypersplenism" associated with hypogammaglobulinæmia (Case 36). There is slight anisocytosis and poikilocytosis. ×700.

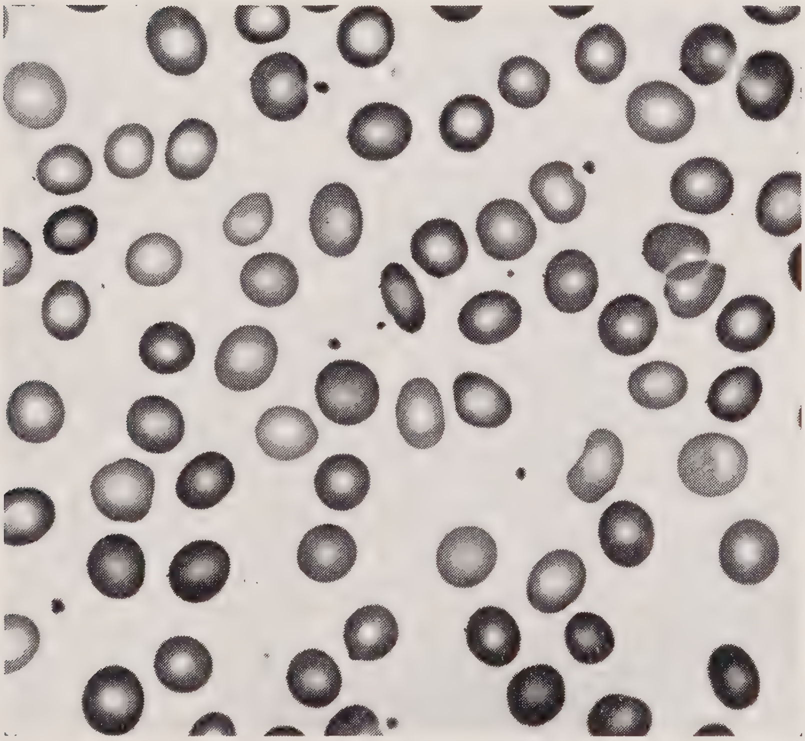

FIG. 238. Photomicrograph of a blood film of a patient thought to be suffering from "hypersplenic hæmolytic anæmia" (Case 37). There is slight anisocytosis, macrocytosis and ovalocytosis. ×700.

To face p. 936.

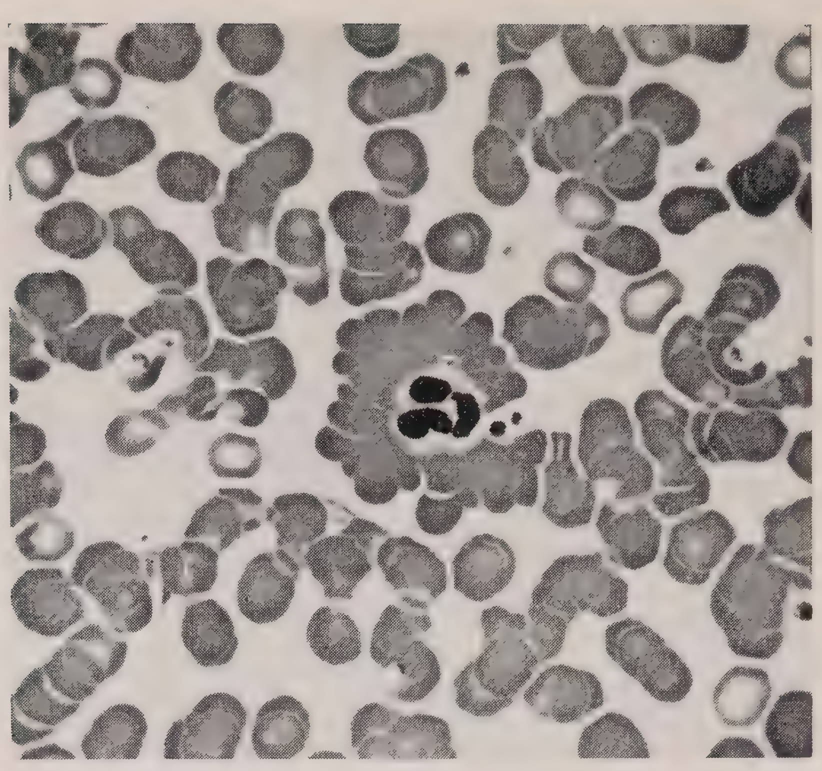

FIG. 239. Photomicrograph of a blood film of a patient thought to be suffering from "hypersplenic hæmolytic anæmia" (Case 37). A neutrophil is surrounded by a "rosette" of erythrocytes apparently adhering to it. ×700.

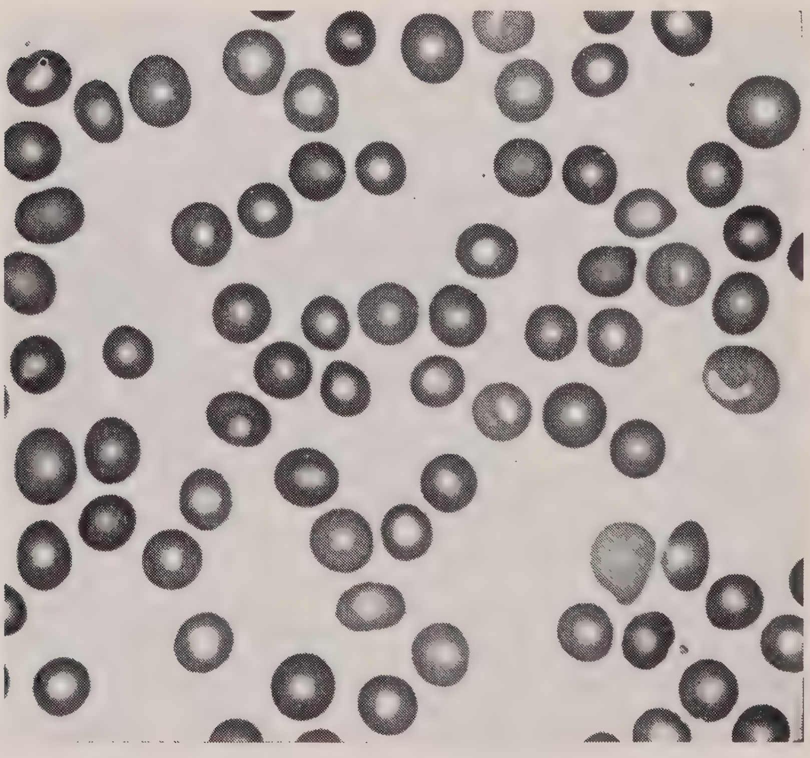

FIG. 240. Photomicrograph of a blood film of a patient suffering from hæmolytic anæmia of unknown type (? "hypersplenic hæmolytic anæmia") (Case 38). There is slight anisocytosis and macrocytosis, but no obvious spherocytosis and little poikilocytosis. ×700.

or lympho-proliferative disease (p. 768), reticuloses (p. 756) and liver disease (p. 836). This is thought to happen, too, in the storage diseases, *e.g.*, Gaucher's disease (Merskey and Budtz-Olsen, 1953; Medoff and Bayrd, 1954; Giblett *et al.*, 1956).

"HYPERSPLENIC HÆMOLYTIC ANÆMIA"

Cases of hæmolytic anæmia exist, rather rarely, which appear to be acquired but which do not fit easily into any of the well known categories; in particular, all tests in these patients for the presence of auto-antibodies are consistently negative and no underlying systemic disorder appears to be present. In the first edition of this book (Dacie, 1954, p. 375) the possible existence of "hypersplenic hæmolytic anæmia" was discussed, a case report of Dausset, Paraf and Caroli (1952) was referred to and two patients whose hæmolytic anæmia was of unknown origin, but could perhaps be attributed to a type of hypersplenism, were described (Dacie, 1954, Cases 29 and 30). A similar obscure case in which splenectomy led to remission was reported by Lind (1955).

Several further patients whose hæmolytic anæmia has been equally mysterious and unclassifiable have been studied by the author in recent years. Two patients of this type are described below (Cases 37 and 38). In both, some evidence was obtained which suggested that an enlarged spleen was playing a large part in bringing about the hæmolysis: both patients showed a marked in-vivo accumulation of radioactivity in the spleen following the labelling of erythrocytes with ^{51}Cr and the patient who was submitted to splenectomy responded excellently to the operation. If these patients were in fact suffering from a type of hypersplenism, then it has to be admitted that it is not easy to understand why the patients' erythrocytes were being destroyed to an important degree at a time when the leucocyte and platelet counts were relatively little or not at all affected. The hæmatological findings are clearly more in keeping with the spleen removing erythrocytes which had been damaged or altered in some way; the difficulty is that no evidence whatever for the existence of an erythrocyte abnormality could be obtained by currently available in-vitro tests.

Case Report: "Hypersplenic Hæmolytic Anæmia"; (?) "Primary Hypersplenism"

Case 37. The patient, W. Y., was a male aged 37, a miner by occupation. He had been well until November 1961, 18 months before investigation, when he developed a transient attack of jaundice which lasted only a few days. Six months later he became jaundiced again, and a third attack of jaundice was noticed in February 1963. This time he felt unwell and lacked energy. He had not noticed any abnormal colouration of urine or fæces. There was no family history of jaundice or anæmia. He was referred by Dr. R. D. C. Johnstone and admitted to Hammersmith Hospital for investigation in April 1963.

Physical Examination. He was found to be slightly jaundiced and looked anæmic. The spleen was palpable 5 cm. below the left costal margin and was firm in consistency. No other abnormal physical signs were elicited; in particular, the liver was not palpable and the peripheral lymph nodes were not significantly enlarged.

Laboratory Findings. The hæmoglobin (range of four observations) was 9·6–10·2 g. per 100 ml.; the erythrocyte count was 2,400,000–2,700,000 per cu. mm., with 13–19% reticulocytes; the MCV averaged 126 cu.μ; the leucocyte count was 4,000–6,000 per cu. mm., with 62–82% neutrophils (2,480–4,920 per cu. mm.), and the platelet count was 147,000–185,000 per cu. mm.

The erythrocytes were mostly rounded normocytes or macrocytes, with slight anisocytosis and ovalocytosis. Polychromasia was quite conspicuous but there was no spherocytosis (Fig. 238). The leucocyte alkaline phosphatase score was normal (28–71 units). The serum bilirubin was 1·2–1·4 mg. per 100 ml.; serum alkaline phosphatase was 8 K.A. units, thymol turbidity 0 units and zinc sulphate precipitation 3 units. The urine contained an excess of urobilinogen but no bile pigment. No hæmosiderin was detected in the urine deposit.

Serology. The direct antiglobulin test was negative using a broad-spectrum antiglobulin serum at a range of dilutions and no abnormal antibodies could be demonstrated in the patient's serum using papainized erythrocytes. The cold-agglutinin titre was 4 at 4° C. The acidified-serum test was negative.

Further Investigation. The erythrocyte osmotic fragility was normal: initial lysis 0·5% NaCl.; MCF 0·43% NaCl. After incubation at 37° C. for 24 hours, the majority of the cells were abnormally resistant. The rate of autohæmolysis was normal: there was 2·6% lysis at 48 hours, which was reduced to 0·25% lysis in the presence of glucose.

Glucose consumption and pyruvate kinase, G-6-PD and 6-phosphogluconic dehydrogenase activities were approximately twice normal, which was consistent with the presence of a young-cell population.

Second Admission. The patient was readmitted to Hammersmith Hospital in March 1964 for further studies. He had experienced considerable breathlessness on exertion and had attacks of nausea and vomiting.

The spleen was now palpable 7 cm. below the left costal margin. A cholecystogram demonstrated the presence of non-opaque calculi.

He was found to be rather more anæmic than previously and to have a slight leucopenia also. The hæmoglobin ranged between 7·2 and 8·9 g. per 100 ml., with 14–30% reticulocytes; the leucocyte count varied between 2,300 and 4,000 per cu. mm., with 54–86% neutrophils (1,510–3,320 neutrophils per cu. mm.) and the platelet count varied between 119,000 and 260,000 per cu. mm. On several occasions neutrophils appeared in fixed blood films to be surrounded by a rosette of erythrocytes (Fig. 239). No leucocyte antibodies could be demonstrated and the significance of this observation is unknown. LE cells were not demonstrated.

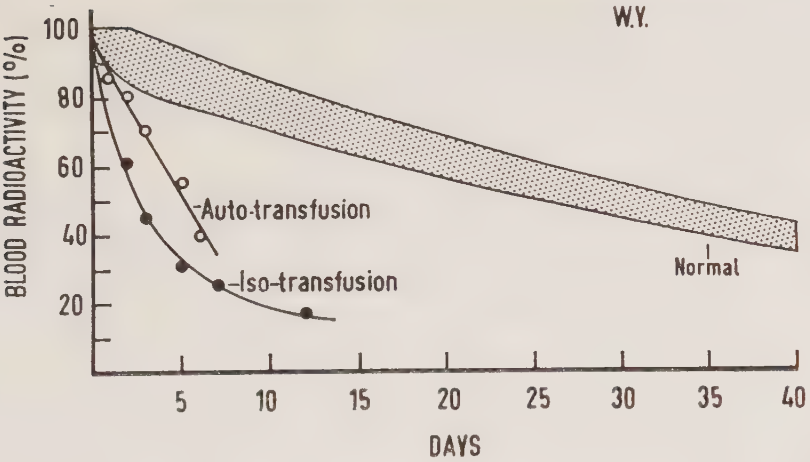

FIG. 241a. Results of ^{51}Cr erythrocyte labelling studies in Case 37. 1: cell survivals.

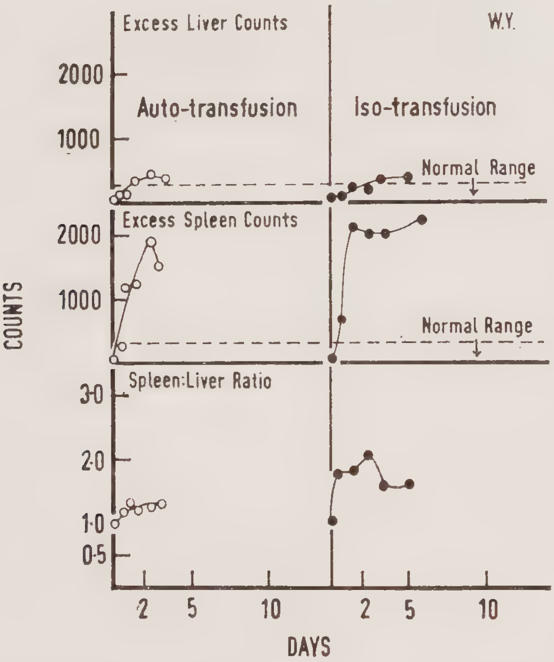

FIG. 241b. Results of ^{51}Cr erythrocyte labelling studies in Case 37. 2: surface counting figures.

Serum proteins were 6·6 g. per 100 ml., albumin 4·4 g. and globulins 2·2 g.; the electrophoretic pattern was normal. The serum bilirubin was 2·2 mg. per 100 ml. Bromsulphthalein retention was normal (5%). The serum iron was 82 μg. per 100 ml. and total iron-binding capacity 228 μg. per 100 ml. Serum folate was reduced at 3·0 μmg. per ml.

Serological studies were again negative: in particular, the direct antiglobulin and D.A.T. tests were negative.

The results of osmotic fragility studies were the same as on the previous admission but autohæmolysis was slightly increased; there was 4·9% lysis at 48 hours, reduced to 0·8% lysis in the presence of glucose.

^{51}*Cr Studies.* The patient's own erythrocytes were labelled. They were rapidly eliminated: the ^{51}Cr T$\frac{1}{2}$ was 5 days and there was a marked accumulation of radioactivity in the spleen and a very slight excess in the liver. The test was repeated with normal compatible erythrocytes: these were eliminated even faster: the ^{51}Cr T$\frac{1}{2}$ was 2·5 days and again there was a marked excess of radioactivity in the spleen and a minimal excess in the liver (Fig. 241a, b).

Splenectomy. It seemed clear that the spleen was a major factor in the patient's hæmolytic anæmia and in the absence of any reasonable alternative a tentative diagnosis of "primary hypersplenism" was made, with excess hæmolysis the main feature. Splenectomy was advised and carried out successfully at Burton-on-Trent.

The patient was seen 4 months after the operation, through the courtesy of Dr. R. D. C. Johnstone. He appeared to be quite well and had lost his jaundice. The hæmoglobin was 13·9 g. per 100 ml., with 0·4% reticulocytes; there were 11,000 leucocytes per cu. mm., 68% neutrophils, and 319,000 platelets per cu. mm. Stained films showed, as before, a few rosettes of erythrocytes surrounding neutrophils.

Sections of the spleen were kindly supplied by Dr. van der Merwe. These showed that the lymphoid follicles were essentially normal in size, number and structure. The reticulum cells of the red pulp and the littoral cells of the sinuses were unusually conspicuous and the pulp was infiltrated by scattered neutrophils and a few eosinophils. Erythrophagocytosis was not easily seen but the pulp contained a great excess of iron.

Summary. The patient developed an apparently acquired moderately severe hæmolytic anæmia associated with moderate splenomegaly and slight leucopenia. Serological studies were negative and in the absence of evidence for any other disorder hypersplenism was diagnosed. Splenectomy was carried out. Four months later the patient appeared to be well and there were no signs of continuing hæmolysis.

Case Report: Chronic Hæmolytic Anæmia, probably Acquired, of Undiagnosed Type. (?) "Hypersplenic Hæmolytic Anæmia"

Case 38. The patient, J. L., a man aged 43, was referred to the author in October 1959 by the late Dr. Philip Ellman. In April 1959 he had an attack of diarrhœa and vomiting and this led to him being admitted to Hospital where he was found to be pale and breathless and to have proteinuria. He was found to be anæmic: the hæmoglobin was 7·8 g. per 100 ml. and there were 19% reticulocytes. Acquired hæmolytic anæmia was diagnosed and therapy with prednisone, 40 mg. daily, was commenced; later this was reduced to a maintenance dose of 25 mg. a day. The hæmoglobin appeared to rise but by October 1959 it had fallen back to its original level. Between April and October 1959 he had been transfused twice but this had produced only transient rises in hæmoglobin. Mild diabetes had also developed. His spleen was palpable, but otherwise physical examination was essentially negative.

He appeared to have had no significant previous illnesses and he gave no history suggestive of anæmia or jaundice prior to April 1959. He was unmarried. The family history, too, was negative for anæmia and jaundice. No relatives have, however, been examined.

Hæmatological Findings (October 1959). The hæmoglobin was 9·9 g. per 100 ml., erythrocyte count 3,300,000 per cu. mm., MCV 104 cu.μ, MCHC 28% and reticulocyte count 23%. The leucocyte count was 11,000 per cu. mm. and the platelet count 212,000 per cu. mm. Stained films showed slight anisocytosis and macrocytosis, considerable polychromasia but little poikilocytosis, and no obvious spherocytosis (Fig. 240).

The serum bilirubin was 1·2 mg. per 100 ml. Hæmoglobin electrophoresis gave a normal AA pattern and Hb-F was not detectable. Osmotic fragility was very slightly increased: lysis commenced at 0·525% NaCl and the MCF was 0·46% NaCl; after incubation the result was essentially normal, lysis commencing in 0·70% NaCl and the MCF was 0·60% NaCl. The rate of autohæmolysis was normal: 1·4% lysis without glucose, 0·8% with glucose. The acidified-serum test was negative.

The direct antiglobulin test was negative and no abnormal antibodies could be demonstrated in the patient's serum. The serum complement level was normal (100 units).

The survival of the patient's own erythrocytes was studied using ^{51}Cr. This showed a rapid rate of destruction and a marked accumulation of radioactivity in the spleen: the ^{51}Cr $T\frac{1}{2}$ was 7·5 days, and the mean cell life-span was estimated to be about 14 days.

No exact diagnosis could be made as the result of these studies, but in view of the clear evidence of accumulation of ^{51}Cr in the spleen splenectomy was advised. The patient, however, felt reasonably well and managed to continue working, and was not willing for splenectomy to be carried out. The prednisone was tailed off and eventually discontinued.

Further Progress. The author has seen this patient at various times from 1959 until the time of writing. He has felt well but has remained slightly anæmic and signs of hæmolysis have persisted: the hæmoglobin has usually been about 12 g. per 100 ml. and the reticulocyte count has ranged from 8 to 12%. The leucocyte and platelet counts have been normal. The spleen has remained palpable and the mild diabetes has persisted.

When last seen in May 1966, 7 years after the apparent onset of his illness, the hæmoglobin was 12·6 g. per 100 ml., erythrocyte count 4,000,000 per cu. mm., MCV 100 cu.μ, and reticulocyte count 12·4%. The leucocyte count was 6,000 per cu. mm. and the platelet count 183,000 per cu. mm.; leucocyte alkaline phosphatase was normal.

As before, the direct antiglobulin test was negative and no abnormal antibodies could be demonstrated in the serum. The osmotic fragility and autohæmolysis tests gave normal results; the serum bilirubin was 1·3 mg. per 100 ml.

Erythrocyte pyruvate kinase activity was slightly raised as was glucose consumption. The glutathione content (74 mg. per 100 ml.) and percentage of unstable glutathione (9%) were normal, and a screening test for G-6-PD deficiency also gave a normal result (40 minutes). The acidified-serum test was negative and the urine was free from hæmosiderin.

Physical examination revealed nothing abnormal except that the spleen was palpable 1 cm. below the left costal margin and the liver 1–2 cm. below the right costal margin.

Summary. The patient has an apparently acquired hæmolytic anæmia of undiagnosed type. The spleen, at least, appears to be an important hæmolytic organ. All tests for auto-antibodies and for intrinsic erythrocyte abnormalities have given negative results. Mild hæmolytic anæmia persists 6 years after its apparent onset. The possibility that the hæmolytic anæmia was a manifestation of an underlying disease of the lymphoreticular system was at first seriously considered but as the years pass this would appear to be less likely.

It is admittedly difficult to prove or disprove the possibility that this patient is suffering from a congenital hæmolytic anæmia (of obscure type) which was first brought to light by an infection (in 1959). Study of the survival of normal erythrocytes in the patient's circulation which could provide indirect evidence on this point has, however, not been carried out. His further history is being watched with great interest.

INCREASED HÆMOLYSIS IN ASSOCIATION WITH VITAMIN DEFICIENCIES

Brief descriptions of the effect of deficiencies of vitamin B_{12}, folic acid, vitamin C and vitamin E on the life-span of the erythrocytes are given below.

Vitamin-B_{12} and Folic-Acid Deficiency; Pernicious Anæmia

It is generally agreed that there is an important hæmolytic component in severe megaloblastic anæmia whether this is caused by deficiency of vitamin B_{12} or folic acid. Thus, clinical jaundice is a well known phenomenon in severely affected patients and where studies of fæcal urobilinogen excretion have been carried out values greatly exceeding the normal have been found.

Urobilinogen Excretion Studies. A number of important studies on urobilinogen excretion were carried out as far back as the 1930's in patients suffering from pernicious anæmia (P.A.) (Farquharson, Borsook and Goulding, 1931; Filo, 1931; Watson, 1931; Barker, 1938). Farquharson, Borsook and Goulding, in particular, who also discussed earlier views on the question of hæmolysis in pernicious anæmia, published a mass of detailed observations on 12 patients: they showed that the amount of urobilinogen in the fæces might equal that derived from 8–13 g. of hæmoglobin daily, which amounted in some cases to more than one-tenth of the calculated total circulating hæmoglobin. They found that the urine urobilin concentration was only likely to be markedly raised in very ill patients, and they attributed this, at least in part, to a failure of liver function. Following effective therapy with liver, the fæcal urobilinogen levels (and the serum bilirubin) were found to return quickly to normal. Farquharson and his colleagues concluded that the disturbance in pigment metabolism was probably due to an abnormality of the erythrocytes which led to their early destruction, *i.e.*, they considered the hæmolysis to be a secondary event, an interpretation which is now generally accepted.

At the present time, in severe megaloblastic anæmia, it is believed that much of the excess hæmolysis takes place within the bone marrow ("ineffective erythropoiesis") and not only after the erythrocytes have been liberated into the blood stream.

Erythrokinetics in Pernicious Anæmia. The evidence for intramedullary hæmolysis has been based on erythrokinetic measurements employing isotopes. Thus, London and West (1950), using ^{15}N, showed that as much as 40% of the fæcal urobilinogen might be derived from sources other than the circulating blood. Radioactive iron has, however, been more widely used and it is the studies with ^{59}Fe which have more than anything else demonstrated the extent and importance of the ineffective erythropoiesis in pernicious anæmia. In particular, these studies have demonstrated the wide discrepancy which exists between the utilization of iron calculated from plasma iron turnover (which is increased compared to the normal) and the effective utilization of this iron as measured by its incorporation into circulating hæmoglobin (which is decreased compared to normal) (Finch *et al.*, 1956; Pollycove, 1960; Myhre, 1964).

That excessively rapid extra-medullary hæmolysis also takes place in pernicious anæmia has been established by measurements of erythrocyte life-span.

First, some important studies of erythrocyte life-span were undertaken using the Ashby method. Ashby (1921b) in fact carried out some of her extensive and pioneer studies on patients suffering from pernicious anæmia. Generally, she found that the transfused group-IV (O) blood survived at least as long in pernicious-anæmia patients as in normal or other control subjects, but by studying the total erythrocyte count also she deduced that "definite" increases in the rate at which the patients' own erythrocytes were being destroyed took place from time to time in a minority of her patients. Twenty-five or more years later, Loutit (1946) and Singer, King and Robin (1948) showed that the erythrocytes of a small number of patients with pernicious anæmia had a moderately reduced life-span after transfusion to normal recipients.

More extensive studies using the Ashby technique were carried out by Hamilton, DeGowin, Sheets, Janney and Ellis (1954) and Hamilton, Sheets and DeGowin (1958). An intrinsic erythrocyte defect as well as an extra-corpuscular hæmolytic mechanism were postulated and there were indications that the random destruction was abolished if vitamin B_{12} was given before an experimental transfusion of normal erythrocytes. Hamilton, Sheets and DeGowin (1958) found, too, that incubation *in vitro* of normal cells in P.A. plasma could be deleterious in that the survival of the cells was impaired when they were retransfused into normal subjects.

The above-quoted studies were all carried out on patients with typical pernicious anæmia due to vitamin-B_{12} deficiency. Comparable studies in patients with megaloblastic anæmia due to folic-acid deficiency are awaited. The preliminary data of Hollingsworth and Adams (1955) suggested that transfused normal erythrocytes may in fact become irreversibly damaged in the circulation of a patient who is deficient in folic acid.

There appear to be but few reports of studies carried out using radioactive chromium. However, Finch and his co-workers (1956) studied two patients and reported ^{51}Cr half-times of 16 and 18 days, respectively, equivalent to a three-times increase in the rate of destruction. On the other hand, Brante (1956) reported that there was no increase in the rate of elimination of normal transfused erythrocytes in four patients within the first 10 days; this was followed in two of them, however, by an apparent acceleration of elimination following the start of treatment. The practical difficulties of carrying out reliable measurements in patients whose blood status is not in equilibrium and who may need to be treated urgently need no emphasis.

An interesting and comprehensive study was published by Hallberg (1955), who reviewed earlier findings on the excretion of bile pigments in pernicious anæmia and reported on personal studies using carbon monoxide excretion as an index of hæmoglobin metabolism. An increased CO concentration in the expired air was found in 25 out of 28 cases of pernicious anæmia, but although the CO concentration fell when the patients responded to treatment there appeared to be no clear relationship between CO excretion and the severity of a patient's anæmia. Hallberg concluded that the viability of P.A. erythrocytes varied widely and that much of the excess hæmolysis was due to the destruction of cells which had a very short (*i.e.*, 1-day) life-span.

As in other hæmolytic anæmias, the normal plasma haptoglobins are usually absent in untreated pernicious anæmia. Nyman (1957) reported the results of serial studies in eight patients with pernicious anæmia. Before treatment methæmalbumin was demonstrated in the sera of seven of the eight patients and haptoglobins were absent in all. Within 3–6 days of the beginning of treatment the methæmalbumin disappeared and traces of haptoglobins reappeared; they reached their normal level in 2–3 weeks. Similar observations were made by Neale, Aber and Northam (1958) in patients with pernicious anæmia and with megaloblastic anæmia due to folic-acid deficiency.

The excess hæmolysis of severe megaloblastic anæmia has without doubt a complicated origin. But, primarily, it would seem to be the result of the grossly abnormal erythropoiesis and the consequent intra-medullary death of many of the developing erythroblasts (and the subsequent excretion of the liberated hæm as urobilinogen). On top of this, it seems likely that many of the abnormal erythrocytes which are produced have a reduced viability. The presence of cell fragments in peripheral blood films is probably good evidence for this and it seems likely that a significant proportion of this extra-medullary hæmolysis takes place in the spleen (Pollycove, 1960). Whether deficiency of vitamin B_{12} or folic acid leads to increased hæmolysis independently of their effect on erythropoiesis is less certain. An auto-immune hæmolytic mechanism in pernicious anæmia seems unlikely; although cold auto-agglutinins at raised titres (Cohen and Jones, 1924) and positive antiglobulin tests (Selwyn and Alexander, 1951; Forshaw and Harwood, 1965) have been described, the antibodies do not seem to have been of significance in relation to hæmolysis.

Forshaw and Harwood (1965) reported that positive direct antiglobulin reactions can frequently be obtained in patients with megaloblastic anæmia due either to vitamin B_{12} or folic-acid deficiency. Positive reactions were obtained in 21 out of 59 patients; but most of the reactions were weak ones and all that were tested were found to be of the non-γ type. The significance of these reactions is unclear; they became negative 1–3 weeks after starting treatment, except in one case.

Vitamin-C Deficiency

There is some evidence that increased hæmolysis may play a part in the anæmia of scurvy. Thus Vilter, Woolford and Spies (1946) in a study of 19 patients reported that the fæcal urobilinogen excretion was increased in three of them, that there might be slight or moderate jaundice and that raised reticulocyte counts were common. They considered that it was unlikely that these signs, pointing to hæmolysis, could be completely explained by the occurrence of hæmorrhage into the tissues and the absorption of blood pigment from ecchymoses.

Merskey (1953) made a series of measurements of the survival of normal erythrocytes in scorbutic patients using the Ashby method and obtained evidence for an increased loss of the normal transfused cells in 14 out of 16 patients. Once more, this did not seem to be accountable by blood loss.

The nature of the possibly hæmolytic component to the anæmia of scurvy remains uncertain. It is possible that the erythrocytes are intrinsically defective, as Kisch and Martin (1957) have described vacuoles demonstrable by electron microscopy in a small proportion of the erythrocytes of scorbutic guinea-pigs.

Vitamin-E Deficiency

There is no substantial evidence that deficiency of vitamin E causes increased hæmolysis in man, although it has been shown that the vitamin and its analogues can exert a protective effect in certain in-vitro and in-vivo hæmolytic systems in experimental animals (György and Rose, 1948, 1949; Christensen *et al.*, 1955–6; Allison, Moore and Sharman, 1956). In man, too, in-vitro effects have been demonstrated. Thus, Mackenzie (1954) demonstrated a relationship between the serum tocopherol levels of premature infants and the sensitivity of their erythrocytes to hæmolysis by hydrogen peroxide, and substantially similar results were obtained by Gordon, Nitowsky and Cornblath (1955), who also demonstrated that the susceptibility of the erythrocytes of children suffering from the malabsorption syndrome to hydrogen peroxide was markedly increased.

More recently, Mengel, Kann and Horton (1964) and Mengel, Kann, Smith and Horton (1964) have shown that vitamin-E-deficient mice are more sensitive than normal mice to the hæmolytic effect of hyperbaric oxygen and ultraviolet irradiation (see p. 949). *In vitro*, their erythrocytes were found to be more sensitive to lysis by H_2O_2 and to form greater than normal amounts of lipid peroxides when exposed to the hyperbaric oxygen.

DIET AND EXCESS HÆMOLYSIS

Little is known of the effect, if any, that dietary habit has on the life-span of the human erythrocyte. However, it is widely recognized that severe megaloblastic anæmia (which could be of dietary deficiency origin) is associated with increased hæmolysis (see p. 942), and it has been postulated that this happens, too, in vitamin-C deficiency (p. 945), and possibly also in severe iron deficiency (see below).

Lipids and Cholesterol and Hæmolysis

In animals, the effect of variation in the lipid or cholesterol content of the diet has been extensively studied experimentally and there is consequently quite a large literature on the subject. Thus, Priest and Normann (1962) reported that rats fed on a diet rich in saturated fat and cholesterol developed excess intravascular hæmolysis and renal hæmosiderosis. Excess cholesterol feeding alone, too, is associated with hæmolysis, and Pinter and Bailey (1961), on the basis of crossed-transfusion experiments in rabbits, concluded that a high cholesterol diet led to an intrinsic type of erythrocyte abnormality. More recently, Silver, McMillan and Silver (1964) reported that the hæmolytic anæmia which they produced in rabbits was accompanied by morphological changes in the animals' erythrocytes reminiscent of those seen in human acanthocytosis. Hestorff, Ways and Palmer (1965), who also fed rabbits with cholesterol, reported that their erythrocytes became distorted and contained vacuoles. Erythrocyte survival was impaired *in vivo* and the rate of autohæmolysis *in vitro* was increased to 4–5 times the normal.

References to early work on the hæmolytic anæmias which have been produced in various types of laboratory animals from fat or cholesterol feeding may be found in the above-quoted papers.

Protein Deficiency and Hæmolysis

There is also literature concerning the effect of protein deprivation on hæmolysis in experimental animals, and in a recent paper Delmonte, Aschkenasy and Eyquem (1964) concluded that lack of protein led to the erythrocytes becoming unusually sensitive in various types of lytic systems, as the result, it was thought, of an acquired structural defect.

HÆMOLYSIS IN IRON DEFICIENCY

The often markedly bizarre blood picture and the apparent evidence for erythrocyte fragmentation which is characteristic of severe iron-deficiency anæmia, as well as the studies which have demonstrated increased hæmolysis in severe cases of Mediterranean anæmia where the blood picture is not dissimilar (see Part I, p. 220), have prompted investigation into the role, if any, that increased hæmolysis plays in severe iron-deficiency anæmia.

The early data of Brown, Hayward, Powell and Witts (1944) demonstrated that normal erythrocytes usually survived normally or almost normally in patients with moderately severe hypochromic anæmia. However, these results are not necessarily relevant to the survival of the patient's own cells in his own circulation, for which auto-survival studies using ^{51}Cr are required. A small number of such studies have in fact been carried out but, unfortunately, the results so far available have been somewhat conflicting.

In infants, Rasch, Cotton, Griggs and Harris (1958) studied 14 iron-deficient infants and five control infants and found that the ^{51}Cr $T\frac{1}{2}$ averaged 17 and 32 days, respectively, in the two groups. The apparent impaired survival in the iron-deficient infants did not seem to be explained by a small loss of radioactivity in the stools.

In adults, Verloop, van der Wolk and Heier (1960) reported slight shortening in the ^{51}Cr $T\frac{1}{2}$ in a group of five women with iron-deficiency anæmia and evidence for a major degree of hæmolysis in two additional women who had a severe iron-deficiency anæmia of obscure type. On the other hand, Temperley and Sharp (1962), in a more recent study of 13 patients, failed to obtain evidence for increased hæmolysis in any of them.

Recently, the results of an extensive study of anæmia in hookworm infection have been published by Layrisse, Linares and Roche (1965): the ^{51}Cr $T\frac{1}{2}$ ranged between 6 and 22 days in 31 patients, and it did not seem possible to explain the markedly shortened survival times on blood loss alone. A shortened survival was similarly demonstrated in four infected subjects using the DF^{32}P technique, and blood from an infected (iron-deficient) individual was found to have the same reduced survival when transfused to a normal recipient as it had in the patient's circulation. Layrisse and his colleagues concluded that minor degrees of increased hæmolysis are characteristic of iron-deficiency anæmia (and that in hookworm infection the parasite *per se* does not cause hæmolysis).

Hæmolysis in Copper Deficiency

There appears to be no data on the effect of copper deficiency in man, but increased hæmolysis has been demonstrated under these circumstances experimentally in pigs (Bush *et al.*, 1956). (The occurrence of hæmolytic anæmia in cases of Wilson's disease has been referred to on p. 833.)

HÆMOLYTIC EFFECT OF RADIATION

The difficult question as to whether irradiation of a patient suffering from a chronic lymphoma can precipitate an attack of hæmolysis has already been mentioned (p. 772). In this section brief reference will be made to the possibility that the erythrocytes themselves can be significantly damaged by radiation.

In vitro, it is known that very large doses of radiation indeed are required to produce detectable changes: thus it was only after doses exceeding 20,000 r. that Davis, Dole, Izzo and Young (1950) were able to demonstrate any changes at all, and then in only one test—the mechanical fragility after incubation.

In vivo, Davis and his co-workers found that the excretion of bilirubin was increased in renal-bile-fistula dogs following whole-body irradiation, but they were unable to decide whether this was due to destruction of erythrocyte precursors in the marrow, destruction of circulating erythrocytes or failure of marrow cells to utilize hæmoglobin precursor substances.

A more recent detailed report is that of Stohlman, Brecher, Schneiderman and Cronkite (1957). In dogs submitted to whole-body irradiation (180–600 r.), these authors obtained evidence for damage to circulating erythrocytes, which was considered to be indirect and progressive, and also for the impaired survival of erythrocytes which had become extravasated after irradiation and had re-entered the circulation, as well as for altered chromium elution. In dogs, therefore, significant diminution in erythrocyte survival seems to follow whole-body irradiation at least in the near-lethal range of dosage. Hill (1962), too, working with mice exposed to 550 r. whole-body irradiation found that their erythrocytes subsequently tended to undergo spontaneous auto-agglutination in saline and might undergo lysis in diluted acidified serum. It was suggested that these effects might have followed the peroxidation of lipids.

In relation to man, however, the present author knows of no substantial evidence for significant damage to normal erythrocytes irradiated in conventional therapeutic dosage. But this may not be true of abnormal erythrocytes. Waller and Gerock (1964) have, for instance, described a patient with a carcinoma of the body of the uterus who experienced an unexpectedly severe fall in hæmoglobin, attributed to hæmolysis, following radium insertions and X-ray therapy. Later, this patient's erythrocytes were found to contain about half their normal content of glutathione as the result of an inherited defect.

HÆMOLYTIC EFFECT OF HYPEROXIA

Recently, interest has been focused on the possibility that exposure to high oxygen tensions might promote lysis of erythrocytes. Experimentally, Mengel, Kann and Horton (1964) and Mengel, Kann, Smith and Horton (1964) described how mice exposed to hyperbaric oxygen for as little as 2 hours developed hæmoglobinæmia, increased erythrocyte osmotic fragility and reticulocytosis. As already referred to (p. 945), they also observed that the changes were more marked in vitamin-E-deficient mice and that they could be minimized by the administration of α-tocopherol. They suggested that hyperbaric oxygen produced its damage by causing peroxidation of lipid components in the erythrocyte stroma and surface.

Mengel, Kann, Heyman and Metz (1965) subsequently described how a 64-year-old man treated for a recent hemiplegia by hyperbaric oxygen showed signs of hæmolysis subsequently. It appeared that this particular patient had erythrocytes whose lipids underwent peroxidation unusually easily when exposed to hyperbaric oxygen, or, *in vitro*, to hydrogen peroxide.

INCREASED HÆMOLYSIS IN MARROW HYPOPLASIA OR APLASIA

Bone-marrow failure occurring as a complication of hæmolytic anæmia has been referred to in earlier Chapters of this book under a variety of headings (*e.g.*, Part I, p. 110; Part II, p. 381). The present section is concerned with the reverse situation, namely, the occurrence of increased hæmolysis in association with, and complicating, chronic hypoplastic or aplastic anæmia. (The rather special relationship between marrow hypoplasia and paroxysmal nocturnal hæmoglobinuria is considered separately in Chapter 17.)

Early reports of hæmolysis in aplastic anæmia include those of Barker (1938), who in a large study of a variety of blood diseases concluded that a hæmolytic factor was apparently active in rare cases of aplastic anæmia, and Roth and Jasinski (1949), who reported that two out of three patients with panmyelophthisis complicated apparently by increased hæmolysis recovered after splenectomy. More recently, in a report from Japan, Tajima (1960), who used ^{51}Cr, reported that the erythrocyte life-span was diminished in the majority of patients he had studied, and that in a few of them there was a high uptake of radioactivity by the spleen.

Lewis (1962) has published the results of a more definitive study. Using ^{51}Cr, he showed that the erythrocyte survival was less than normal in 14 out of 16 patients (^{51}Cr T$\frac{1}{2}$, 10–19 days) and possibly subnormal in one further patient (^{51}Cr T$\frac{1}{2}$, 24 days). The stools were tested for radioactivity in each case and in 14 of the patients loss of blood into the gastrointestinal tract was shown to be insignificant. In two, however, it was considerable, 30 ml. and 150 ml. daily on an average, and in the latter instance the blood loss could have explained the unusually rapid loss of radioactivity from the circulating blood. Surface counting was carried out in twelve patients and some excess accumulation of radioactivity was demonstrated in the spleen in five.

Fanconi's anæmia. A small number of studies are available which suggest that a minor to moderate increase in hæmolysis is commonly found in Fanconi's anæmia.

Bernard, Mathé and Najean (1958) carried out ^{51}Cr measurements on four patients and found reduced survivals (^{51}Cr T$\frac{1}{2}$, 12–22 days) in all—two patients, however, were suffering from overt hæmorrhage at the time. Debray and Najean (1959) later reported that in one patient an auto-survival study gave a ^{51}Cr T$\frac{1}{2}$ of 17 days although the same patient's erythrocytes transfused to a normal recipient survived normally (^{51}Cr T$\frac{1}{2}$, 30 days); and they concluded that an extra-corpuscular factor was playing a part in the causation of the excess hæmolysis. Sjölin and Wranne (1962) reported ^{51}Cr T$\frac{1}{2}$ values of 20 days and 21 days, respectively, in a small girl studied on two occasions over a period of 4 years. The possibility of an extra-corpuscular hæmolytic factor seemed to receive some support from the observations of Syllm-Rapoport and co-workers (1965) on a girl aged 8 years. The ^{51}Cr T$\frac{1}{2}$ in an auto-survival experiment was 17 days but normal erythrocytes transfused to the patient were eliminated even faster than this.

Gasser (1961) published the results of following the survival by the Ashby method of normal erythrocytes transfused to two patients with Fanconi's anæmia: elimination was complete in 14 days and 42 days, respectively. [The patient who destroyed the transfused blood rapidly had had many transfusions—he was an adult who had received more than 20 l. of blood (Rohr, 1949)—and this result could well have been due to destruction by iso-antibodies.]

The nature of the increased hæmolysis in aplastic anæmia is obscure. It is tempting to regard it as secondary to the dyshæmopoiesis, but the nature of the implied defect(s) in the erythrocytes and the role (if any) of extra corpuscular factors remain obscure.

HÆMOLYSIS IN BURNS

Ever since the early work of Schultze (1865) it has been realized that heating blood at temperatures above 50° C. rapidly induces the formation of spherical forms, fragmentation of the erythrocytes and eventually hæmolysis. Further work, mainly by German authors around the turn of the century, established the basic facts in relation to the blood changes in the experimentally burned animal. Thus, von Lesser (1890) observed hæmoglobinæmia and hæmoglobinuria in burned animals, in normal animals transfused with the blood of a burned animal and in normal animals injected with blood that had been heated to 60–62° C.; while Silbermann (1890) noted a decrease in the erythrocyte resistance to hypotonic saline in burned animals.

This early work has been repeated in more detail in recent years. Ham, Shen, Fleming and Castle (1948) defined the sequence of changes affecting the red cells and the exact degree of heating necessary to produce them. Using both human and dog erythrocytes, they found that at 46° C. no demonstrable changes followed heating for 1 hour, but at 47–50° C. changes occurred which were dependent upon the duration of the heating and the temperature; whilst at 51–65° C. marked changes followed exposure for as little as 1–2 minutes.

According to Ham, Shen, Fleming and Castle, the first discernible alteration produced by heating is the appearance of bud-like excrescences from the surfaces of the erythrocytes; then, multiple buds appear and these become detached. Finally, the intact erythrocytes and the fragments undergo progressive and irreversible sphering and at this stage their osmotic and mechanical fragilities become markedly increased. Ham, Shen, Fleming and Castle also reported on the sequence of events following the intravenous injection into dogs of previously heated blood; in particular, the changes in osmotic fragility, mechanical fragility and plasma and urine hæmoglobin concentrations were studied in great detail.

The introduction of ^{51}Cr as a label for erythrocytes has provided a means by which the survival of erythrocytes, damaged by heat *in vitro*, can be studied *in vivo* following reinjection. Wagner and his colleagues (1962) were able to demonstrate in this way that heat-damaged cells, like chemical-damaged cells, were removed, if damaged to a minor degree, predominantly by the spleen; on the other hand, when the damage was more severe, the liver became increasingly more important.

The results of a further comprehensive study were recently reported by Kimber and Lander (1964), who studied the morphological changes in human blood which follows heating *in vitro* and also the fate of the heated blood (tagged with ^{51}Cr) when reinjected into the donors. They demonstrated a series of changes ranging from the formation of "pyrospherocytes" to fragmented forms and finally to "agglutinated forms", according to the length of time the erythrocytes were heated at 50° C.

When the heated cells were reinjected into the donors, the results obtained were similar to those of Wagner and his colleagues (1962): with 10–20 minutes' heating the damaged cells were taken out of the circulation almost exclusively by the spleen, but with an increasing time of heating more and more of the cells were taken up by the liver and the rate of clearance from the blood was correspondingly increased. In splenectomized subjects the rate of clearance was slow but with sufficient heating there was uptake by the liver.

Kimber and Lander considered that their data were consistent with the findings in burned subjects in whom fragmented forms are cleared rapidly from the circulation while spherocytes circulate for longer periods (see below).

Hæmolysis in Burns in Man

It is only comparatively recently that it has been appreciated that hæmoglobinæmia and hæmoglobinuria may be observed as an early transient but quite frequent phenomenon in human patients who have been severely burned (Shen, Ham and Fleming, 1943; Brown, 1944, 1946; Ham, Shen, Fleming and Castle, 1948).

Shen, Ham and Fleming (1943) noted hæmoglobinæmia and hæmoglobinuria in 11 out of 40 patients: this was gross in nine patients who had suffered 2nd and 3rd degree burns involving 15–65% of the body surface. Varying degrees of increased erythrocyte osmotic and mechanical fragilities were found in six patients, corresponding with the appearance in the peripheral blood of spherocytes, and, shortly after the burning, of small schistocytes (Fig. 4, Part I, p. 22). In the patients who survived, the spherocytes had disappeared from the circulation and the osmotic fragility had returned to normal within 60 hours.

The hæmoglobinuria lasts as a rule for 24 hours at the most, being most intense soon after burning. Occasionally, according to Sevitt (1957), discolouration of the urine is biphasic or intermittent, with the urine colour clearing after a few hours only to be followed by more hæmoglobinuria later. In other patients hæmoglobin appears in the urine for the first time 12–24 hours after burning.

Approaching the subject from a different angle, James, Purnell and Evans (1951), who carried out careful pigment-excretion studies on burned patients, concluded that increased hæmolysis was an important early factor, particularly in patients with third-degree burns affecting more than 20% of the body surface.

The studies mentioned above refer to acute changes, occurring within a day or so of burning; they are without doubt due to the direct hæmolytic effect of heat on the erythrocytes. Anæmia in burned patients, nevertheless, may increase in severity subsequently and can persist for many weeks. The late anæmia of burns undoubtedly has a complex origin (Moore *et al.*, 1946; Sevitt, 1957). Thus, Moore, Peacock, Blakely and Cope (1946) attributed the anæmia to early hæmolysis, erythrocyte destruction by unneutralized (transfused) plasma antibodies, blood loss from open surfaces, infection and disordered erythropoiesis.

In recent years attention has been directed particularly towards assessing the relative parts played by excess hæmolysis and other factors in the causation of anæmia, especially by Topley and her colleagues at the *British Medical Research Council Industrial Injuries and Burns Research Unit* in Birmingham.

Davies and Topley (1956) and Topley and Jackson (1957) described in detail the hæmatological findings in burned patients using, amongst other means, serial measurements of erythrocyte volume and total blood volume: they found that the average loss of erythrocyte volume in the first 12 hours was about 12%—this was attributed to the direct effect of heat and was associated with schistocytosis, spherocytosis, hæmoglobinæmia and hæmoglobinuria. From the 13th to 48th hour the erythrocyte loss continued and in severely burned patients it averaged 30%; while from the third day onwards the erythrocyte volume fell more slowly at a rate of about 1% per day. Topley (1961) later showed that the percentage of heat-affected cells in the patients' blood films on admission could be used as a rough guide to the severity of the subsequent erythrocyte loss. Muir (1961), too, estimated that the early erythrocyte loss amounted to about 10% of the erythrocyte volume in severely burned subjects who survived.

Topley's studies indicate that hæmolysis does not usually play a major role in the anæmia of a burned patient after the first 24 hours. However, the transfusion of large volumes (*e.g.*, three times or more the patient's own plasma volume) of pooled plasma containing iso-agglutinins (particularly anti-A) can be a potent factor in causing hæmolysis of the recipient's erythrocytes if he is group A (Topley *et al.*, 1963). This mechanism seems to be an important cause of "late" hæmoglobinuria, *i.e.*, that which occurs from 12–48 hours after burning, and also of increased erythrocyte osmotic fragility.

Another mechanism of erythrocyte loss—it cannot strictly speaking be described as a hæmolytic mechanism—is loss of cells from the circulation through local vascular congestion and extravasation into the tissues in the neighbourhood of the burnt areas (Ham, 1944; Moritz, 1947; Sevitt, 1957; Topley and Frost, 1963). The extent of such loss is difficult to determine in man, but Topley and Frost (1963), in an experimental study on rats, computed that about half of the erythrocyte loss in the first 24 hours after burning occurred in the tissues in the region of the burn.

Hæmolysis in Massive Injuries

Brief mention will be made of hæmolysis as a factor in the pathogenesis of the severe anæmias which may complicate war wounds and severe civilian injuries. Although hæmorrhage is by far the most important anæmia-producing factor, the severity of blood loss not infrequently seems to be more than can be accounted for on this basis alone. Thus, Crosby and Howard (1954), in studies in men who had received war injuries in Korea, found a remarkable loss of circulating erythrocyte volume where there was much tissue destruction. Although part of this loss could be attributed to extravasation of blood into the damaged areas, it seemed likely that hæmolysis also contributed to this. A further factor in some cases was the use of group-O blood and the subsequent hæmolysis of the recipients' erythrocytes by iso-agglutinins when very large volumes of blood were transfused.

Careful studies have also been carried out on civilian casualties and rises in fæcal urobilinogen excretion (Baar and Topley, 1956), as well as unexplained losses of erythrocyte volume, possibly attributable to hæmolysis, have been demonstrated (Topley and Clarke, 1956). The increased pigment excretion which has been found to follow serious injuries, with or without massive internal hæmorrhage, could be due to absorption of the breakdown products of extravasated erythrocytes: the finding does not necessarily mean an increased rate of destruction of circulating cells.

HÆMOLYTIC ANÆMIA FOLLOWING CARDIAC SURGERY

It has only recently been recognized that intravascular hæmolysis, sometimes of serious degree, may develop as the consequence of operations on the heart.

Rose, Hufnagel, Freis, Harvey and Partenope (1954) appear to have been the first to have noticed the occurrence of anæmia in patients in whom Hufnagel ball valves had been inserted into the thoracic aorta, but they did not discuss the nature of the anæmia nor describe the blood picture.

Sarnoff and Case (1955) reported that all nine patients in whom ball valves had been inserted were slightly to moderately anæmic when examined 20–28 days post-operatively; they made the point that the anæmia could be due to intravascular hæmolysis but added that this had not yet been looked for. Stohlman, Sarnoff, Case and Ness (1956) experimented with dogs and found that intravascular hæmolysis in fact commonly followed the insertion of a lucite conduit containing a Hufnagel ball valve between the left ventricle and thoracic aorta and they postulated that the erythrocytes were damaged by being forced between the rigid ball and the rigid valve casing. They did not study the dogs' blood picture in detail.

Sayed, Dacie, Handley, Lewis and Cleland (1961) published a detailed account of a patient who underwent an operation for the repair of an ostium-primum defect with Teflon and who subsequently developed persistent and severe intravascular hæmolysis. This patient was eventually reoperated on and it was found that a jet of blood, regurgitating through a cleft in the mitral valve, had been impinging on the artificial Teflon septum which had been inserted between the two sides of the heart. An area on this septum was bare of endothelial covering; the area was depressed and had formed a little cul-de-sac into which the jet of blood appeared to be playing. Obliteration of this cul-de-sac and the covering of the area with endothelium was followed by immediate cessation of hæmolysis. It was postulated that this had been brought about by mechanical forces caused by the regurgitant jet of blood being driven against the bare Teflon, but it was not clear whether it was the presence of the Teflon, the lack of endothelial covering or the turbulence of the blood which caused the hæmolysis.

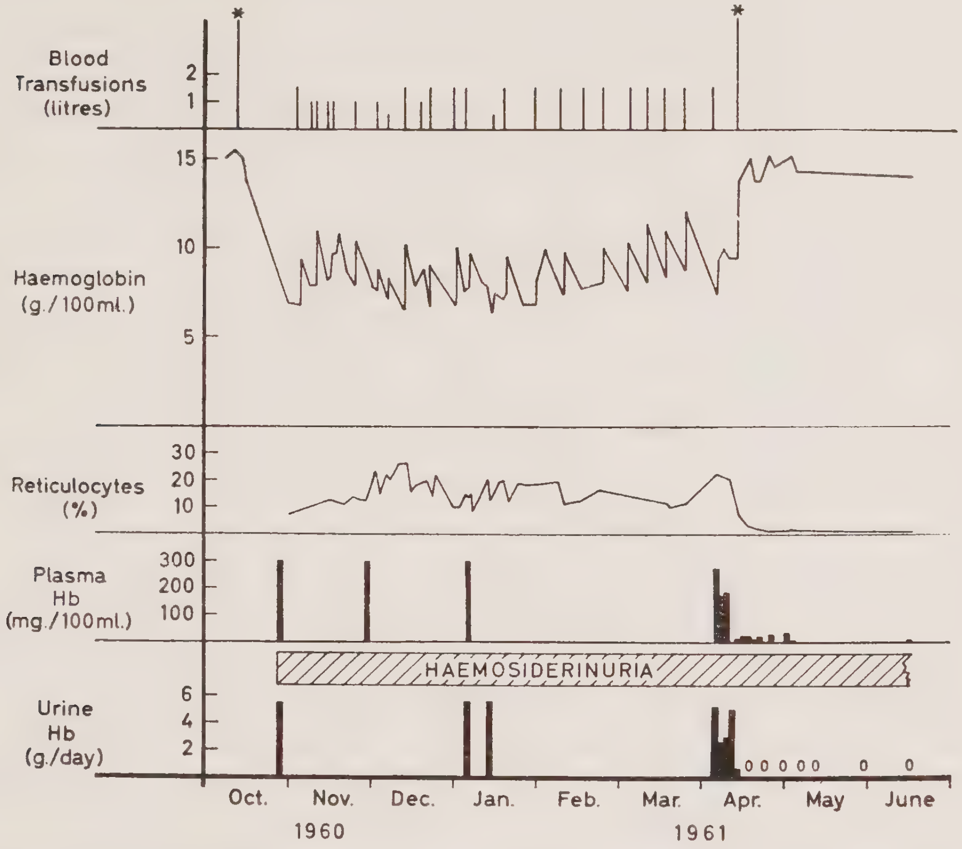

FIG. 242. Hæmatological changes in a patient (A. A.) who had a Teflon graft inserted for an ostium-primum defect. (From Sayed *et al.*, 1961.)

This patient's dramatic course is illustrated in Fig. 242. Anæmia became severe despite a marked reticulocytosis, and he had to be sustained by repeated blood transfusions. Blood films showed striking abnormalities (Fig. 244); in particular, many irregularly contracted and crenated cells, "burr" cells and cell fragments were present, and the appearances were almost, if not quite, indistinguishable from those of microangiopathic hæmolytic anæmia.

^{51}Cr studies showed that normal corpuscles were rapidly destroyed after transfusion to the patient, confirming that the cause of the hæmolysis was not connected with any peculiarity of the patient's own erythrocytes. The leucocyte and platelet counts remained essentially normal throughout the hæmolytic episode. In keeping with the intravascular nature of the hæmolysis were continuously high levels of plasma hæmoglobin (mean 208 mg. per 100 ml), constant hæmoglobinuria (mean 24-hour excretion of hæmoglobin 4·5 g.) and marked hæmosiderinuria (Fig. 245).

Subsequent to the description of Sayed and his colleagues (1961), reports of hæmolytic anæmia following cardiac surgery have been appearing in the literature with increasing frequency (Vandenbroucke, Joosens and Verwilghen, 1962; Sigler, Forman, Zinkham and Neill, 1963; Verdon, Forrester and Crosby, 1963; Stevenson and Baker, 1964; Marsh, 1964a; Brodeur *et al.*, 1964; Gehrmann and Loogen, 1964; Furuhjelm *et al.*, 1964; Sanyl, Polesky, Hume and Browne, 1964; Reed and Dunn, 1964; Sears and Crosby, 1965; Pirofsky, Sutherland, Starr and Griswold, 1965; DeCesare, Rath and Hufnagel, 1965; Yeh, Ellison and Wright, 1965; Viner and Frost, 1965; Petz and Goodman, 1966; Ziperovich and Paley, 1966). It seems in fact that greater or lesser degrees of increased hæmolysis are not uncommon following operations on the heart, particularly those for ostium-primum defects and after aortic-valve replacement. Marsh (1964b), in reviewing the post-operative course of 118 patients, found that two out of 50 patients who had had Teflon septa inserted for ostium-primum defects, six out of 54 patients who had been given aortic-valve prostheses and one out of 14 patients who had been given mitral-valve prostheses developed significant degrees of intravascular hæmolysis as judged by the presence of persistent anæmia, reticulocytosis and hæmosiderinæmia. The above-mentioned patients were all operated on at Hammersmith Hospital, and the series includes the original patient of Sayed and his colleagues, who was, however, the most severely affected.

DeCesare, Rath and Hufnagel's (1965) experience is very similar. Of 32 patients who had had aortic-valve prostheses, six were found to be anæmic. Three of them were found to have overt hæmolytic anæmia and three died before hæmatological studies could be carried out. The three patients who had overt hæmolytic anæmia were reoperated on and in each case the prosthesis was found to have become partly detached.

The frequency of subclinical hæmolysis after certain types of cardiac surgery has been demonstrated by several recent studies, and it has also become apparent that minor degrees of increased hæmolysis are commonly present, too, in unoperated patients suffering from severe disease of the aortic valve (see below and also p. 965).

Brodeur and his colleagues (1964, 1965) reported on 21 patients with aortic-valve disease before they were operated on, on 12 patients who had been provided with aortic prostheses and eight who had had more than one prosthesis inserted. Some degree of subclinical hæmolysis could be demonstrated in almost every case. The mean ^{51}Cr T$\frac{1}{2}$ of each series was 20·7, 20 and 20 days, respectively, compared with a normal ^{51}Cr T$\frac{1}{2}$ of 27 ± 3 days. The minimum packed cell volumes in the series were 30%, 29% and 28% and the maximum reticulocyte counts were 3·4%, 4·5% and 4·3%, respectively.

Dennis and co-workers (1964) studied four patients who received aortic-valve prostheses, two who had mitral-valve replacement and three who had both procedures: minor to moderate reductions in erythrocyte life-span were demonstrated by the ^{51}Cr or DF^{32}P techniques in each case.

Anderson, Gabrielli and Zizzi (1965) have published the results of an important study carried out on 10 patients who had mitral-valve prostheses inserted and on eight patients who had been provided with aortic-valve prostheses 6 months to 2½ years prior to being investigated. All the patients were found to have low serum-haptoglobin levels. (Most had been shown to have normal levels pre-operatively—two had, however, low levels.) The serum lactic dehydrogenase (considered to be derived from hæmolysed erythrocytes) was elevated in all the patients, particularly in those with aortic prostheses. Plasma-hæmoglobin levels were raised in most of the patients with mitral prostheses and in all who had aortic prostheses, and, significantly, further rises followed controlled exercise. Erythrocyte survival was measured in nine patients: in six with mitral prostheses the ^{51}Cr T$\frac{1}{2}$ varied between 15·5 and 26·5 days, and in three with aortic prostheses between 15·5 and 22·0 days. One patient with a mitral prostheses and three who had aortic prostheses were anæmic. The reticulocyte count was slightly raised in most of the aortic cases (maximum 10%) but only in two of the mitral cases (maximum 3·2%). Starr, in discussion, mentioned that in his experience seven patients out of a total of 167 who had had aortic prostheses developed (overt) hæmolytic anæmia.

Some Further Clinical Histories of Patients who have Developed Overt Hæmolytic Anæmia following Cardiac Operations

The history of the patient described by Vandenbroucke, Joosens and Verwilghen (1962), who suffered from Fallot's tetralogy, is remarkable in that intravascular hæmolysis developed following the insertion of a Dacron tube between the aorta and pulmonary artery. When this artificial channel became obliterated by thrombosis the hæmolysis ceased.

Sigler, Forman, Zinkham and Neill (1963) reported on three children with atrio-ventricular canal defects, all of whom had undergone repair operations employing Teflon. All three developed signs of moderately severe intravascular hæmolysis and two were reoperated on, one with success. Both patients were found to have developed regurgitant jets of blood through the mitral valve and in each case the Teflon appeared to be well endothelialized. The third patient improved spontaneously and was not reoperated on. Sigler and his co-workers pointed out that the continuous intravascular hæmolysis and consequent hæmosiderinuria could quickly lead to iron deficiency and erythrocyte hypochromasia.

Verdon, Forrester and Crosby (1963) described two patients, both of whom had ostium-primum defects. They were reoperated on but in neither case was reoperation successful. At necropsy in the first case the mitral valve was found to be scarred and there was a lack of endothelium on portions of the Teflon patch and suture material adjacent to the mitral defect. In the second patient (who died immediately post-operatively) the Teflon patch, although well endothelialized on its right side, was found to be devoid of endothelium on the left; and part of the suture line between the aortic cusp of the mitral valve and the Teflon patch had been detached, allowing a jet of blood to play upon the Teflon patch. This patient had a very severe hæmolytic anæmia and the clinical and pathological condition was very similar to that of the patient of Sayed and his colleagues (1961).

The patients described by Stevenson and Baker (1964), Marsh (1964a) and Reed and Dunn (1964) had had aortic-valve replacements with Starr-Edwards prostheses.

Stevenson and Baker's first patient had three operations on his aortic valve and was left with a moderately severe hæmolytic anæmia which eventually improved spontaneously. Leakage of blood around the insertion of the valve to the aortic wall was thought to be responsible for aortic regurgitation which persisted after the third operation. Their second patient's anæmia may not have been solely mechanical in origin as he developed post-operative bacterial endocarditis also.

Marsh's (1964a) two patients (adult men) had aortic-valve prostheses inserted for calcific aortic stenosis. Both developed moderately severe intravascular hæmolysis for which transfusions were at first required. However, both patients eventually achieved hæmoglobin equilibrium at about 8–9 g. per 100 ml. and with about 15% of reticulocytes (Fig. 243). Both showed marked hæmosiderinuria but neither had overt hæmoglobinuria. The blood film of one of these patients is illustrated in Fig. 246.

Gehrmann and Loogen (1964) described two patients who developed hæmolytic anæmia after aortic-valve replacement by Hufnagel prostheses. The first patient developed severe symptoms 5 months post-operatively and had to be reoperated on. A valve was found to be loose and was replaced. Subsequently, although incompetence did not seem to be as severe, hæmolysis continued. The second patient similarly developed severe symptoms and a loud diastolic murmur (considered to be due to loosening of a valve's attachment) 10 months after an apparently successful operation. However, compensation for the hæmolysis was achieved and a second operation was not undertaken. ^{51}Cr survival studies were carried out, combined with surface counting, and these, by revealing some excess accumulation of radioactivity in the spleen, indicated that hæmolysis was not exclusively intravascular.

Sanyl, Polesky, Hume and Browne (1964) gave an interesting account of a patient who, having had an ostium-primum defect repaired, developed a compensated hæmolytic anæmia subsequently. Then followed an acute episode of cardiac failure which was associated with increased mitral insufficiency and this was accompanied by an exacerbation of the hæmolysis. The cardiac failure appeared to have been precipitated by anæmia consequent on iron deficiency (produced by the hæmosiderinuria). The patient was treated with iron and by blood transfusions and in this way an apparent vicious circle—anæmia leading to cardiac failure, leading to increased mitral incompetence, leading to increased mechanical hæmolysis within the heart, leading to increased anæmia—was broken.

Yeh, Ellison and Wright (1965) described a patient who had a single prosthetic aortic cusp inserted for calcific aortic stenosis. About 1 year later the cusp appeared to rupture and aortic insufficiency accompanied by severe hæmolytic anæmia developed. Replacement of the ruptured cusp with a Starr-Edwards prosthesis eliminated both the aortic insufficiency and the hæmolytic anæmia.

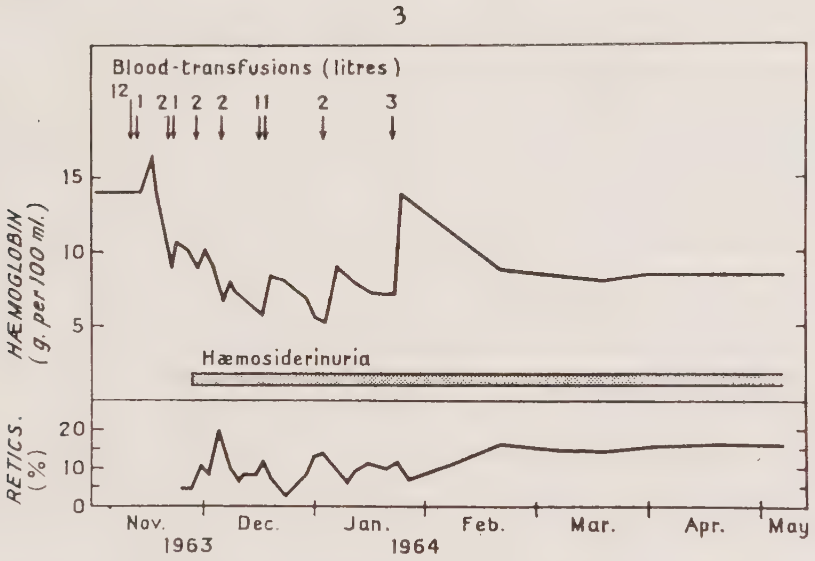

FIG. 243. Hæmatological changes in a patient (A. W.) who had a Starr-Edwards prosthesis inserted for severe calcific aortic stenosis. (From Marsh, 1964a.)

Sears and Crosby (1965) described two cases. The first patient, who had an ostium-primum defect closed with a Teflon patch, became anæmic 2 weeks post-operatively. The ^{51}Cr $T\frac{1}{2}$ was shown to be 8 days and atypical lymphocytes and heterophile agglutinins against sheep cells (absorbed by guinea-pig kidney) were also demonstrated to be present. Later jaundice developed and viral hepatitis was diagnosed; the patient made a gradual recovery. Sears and Crosby noted that the hæmoglobinuria was diurnal—the urine passed in the day was darker than that passed at night or on awakening in the morning; they demonstrated that increased physical activity was accompanied by increased hæmolysis and concluded that the link between physical activity (minimal at rest or during sleep at night) and increased hæmolysis was an increased cardiac output leading to increased mechanical hæmolysis. Sears and Crosby demonstrated that the patient managed to maintain a better hæmoglobin level following transfusion and this they attributed to the rise in hæmoglobin allowing a fall in cardiac output and a diminution in hæmolysis, thus breaking a vicious circle.

Sears and Crosby's second case had a Starr-Edwards prosthesis inserted. This patient, who developed a compensated hæmolytic anæmia of moderate severity, was found to have slightly lower values for plasma hæm pigments when at rest compared to periods of activity. This paper is of particular practical value as it stresses the therapeutic value of rest (to reduce cardiac output), of blood transfusion, and of iron therapy (to combat iron loss in the urine).

DeCesare, Rath and Hufnagel (1965) described three patients who developed intravascular hæmolysis following aortic-valve replacements; they emphasized that in each case the hæmolysis coincided with the development of signs of severe post-operative aortic insufficiency. In each case, on reoperation, the artificial valves were found to have become torn or dislodged.

Pirofsky, Sutherland, Starr and Griswold (1965) described seven patients who developed hæmolytic anæmia following the insertion of a Starr-Edwards aortic-valve prosthesis (with or without replacement of the mitral or tricuspid valves) in whom the hæmolysis did not appear likely to be due to mechanical factors. An auto-immune basis was postulated. In each case signs of excessive hæmolysis developed within 2 weeks of the surgical procedure; this persisted or was relatively transient. In four of the patients signs of the "febrile-lymphocyte-splenomegaly" (post-perfusion) syndrome were also present. Two of the patients appeared to respond well to steroid therapy both clinically and hæmatologically and two others probably responded favourably. Positive direct antiglobulin and/or bromelin tests were given by six out of the seven patients; in general, however, these tests were only weakly or moderately strongly positive. Nevertheless, antibody globulin, which behaved as a panagglutinin and appeared to be identical or very similar to antibodies eluted from other cases of auto-immune hæmolytic anæmia, was eluted from the erythrocytes of two patients. The tests were observed to be positive in the different patients for periods which varied between 1 week and 1 year. Pirofsky and his colleagues suggested that the apparent auto-immune syndrome which they had observed might have been due to mechanical factors damaging the patients' erythrocytes so that surface antigens became unmasked or modified with the result that antibodies were produced against them. An alternative hypothesis which was considered, but which appeared to be a little less likely, was that lymphocytes introduced by the massive transfusions the patients had received had settled down in the recipients and had led to a graft-*v*-host reaction, with the erythrocytes being one of the target organs.

It should be added that Hjelm, Högman, Finnson and Malers (1964) had previously described a patient who developed a direct positive antiglobulin test following the insertion of a Starr valve for mitral insufficiency. Incomplete auto-antibodies, in part anti-e-specific, were present in the serum. There were, however, no clinical signs of erythrocyte destruction.

Petz and Goodman (1966) have recently described two further patients who developed hæmolytic anæmia, thought to be of mechanical origin, following the insertion of Starr-Edwards aortic-valve prostheses. These cases are particularly interesting as ringed sideroblasts (with intra-mitochondrial iron demonstrable by electron-microscopy) were found to be present in bone-marrow films. The changes were dramatic and marked in the first patient but much less so in the second. Petz and Goodman raised the question of the specificity of the sideroblastic change and the relationship between sideroblast formation and overloading with iron. (It may be added that ringed sideroblasts are not typically seen in hæmochromatosis or transfusional siderosis.)

Summary of Blood Changes in the Hæmolytic Anæmias Following Cardiac Surgery

The main features are as follows: anæmia is slight to severe, and is normocytic or slightly macrocytic; in long standing cases hypochromasia may become noticeable as iron deficiency develops. Blood films show a moderate degree of aniso-poikilocytosis and, characteristically, the presence of a few to many distorted erythrocytes and cell fragments—the picture being very similar to that of microangiopathic hæmolytic anæmia. Polychromasia will be present according to the height of the reticulocyte count. Photomicrographs of films showing major changes are illustrated in Figs. 244, 246 and 247.

The leucocyte count is usually normal. The platelet count is usually normal, too (except in the immediate post-operative period), but a few moderately low counts have been recorded. Observations made in the author's laboratory on seven patients are recorded in Table 69.

The direct antiglobulin test is characteristically negative, but, as already described above, some weak or moderately strong positive reactions have been recorded, *e.g.*, by Pirofsky and co-workers (1965).

The plasma-hæmoglobin level is characteristically raised and if hæmolysis is severe it may exceed 100 mg. per 100 ml. Haptoglobins are usually very low or absent. The serum bilirubin is slightly or moderately raised. Hæmoglobinuria will be present in the worst affected patients and may be continuous and severe, as in the patient of Sayed and his co-workers (1961) (Fig. 242). Conspicuous hæmosiderinuria is continuously present (Fig. 245); this is likely to be so even if overt hæmoglobinuria has never occurred.

Table 69

Hæmatological Findings in Seven Patients who Developed Hæmolytic Anæmia after Cardiac Surgery

Patient	Minimum hæmoglobin (g./100 ml.)	Maximum reticulocyte count (%)	Leucocyte count (range) (per cu. mm.)	Platelet count (range) (per cu. mm.)	Nature of illness	When first investigated (time after operation)
A.A.* (Fig. 242)	6·2	26	3,000–14,000	125,000–392,000	Ostium-primum defect; Teflon septum	2 weeks
S.M.	10·0	12	6,000–13,000	183,000–313,000	Fallot's tetralogy; two Teflon patches	2 months
E.T.	5·6	36	3,500–11,000	80,000–360,000	Calcific aortic stenosis; Starr-Edwards prosthesis	2 weeks
A.W.	7·1	23	4,000–8,000	123,000–468,000	Calcific aortic stenosis and incompetence; Starr-Edwards prosthesis	2 weeks
G.B.	10·1	15	6,000–8,000	238,000–246,000	Aortic incompetence; Starr-Edwards prosthesis	3 months
Z.K.	10·3	4	5,000–9,000	210,000–230,000	Aortic stenosis; Starr-Edwards prosthesis	3 weeks
B.B.	6·0	14	4,000–14,000	73,000–257,000	Mitral valve replacement	6 weeks

* Described by Sayed *et al.* (1961).

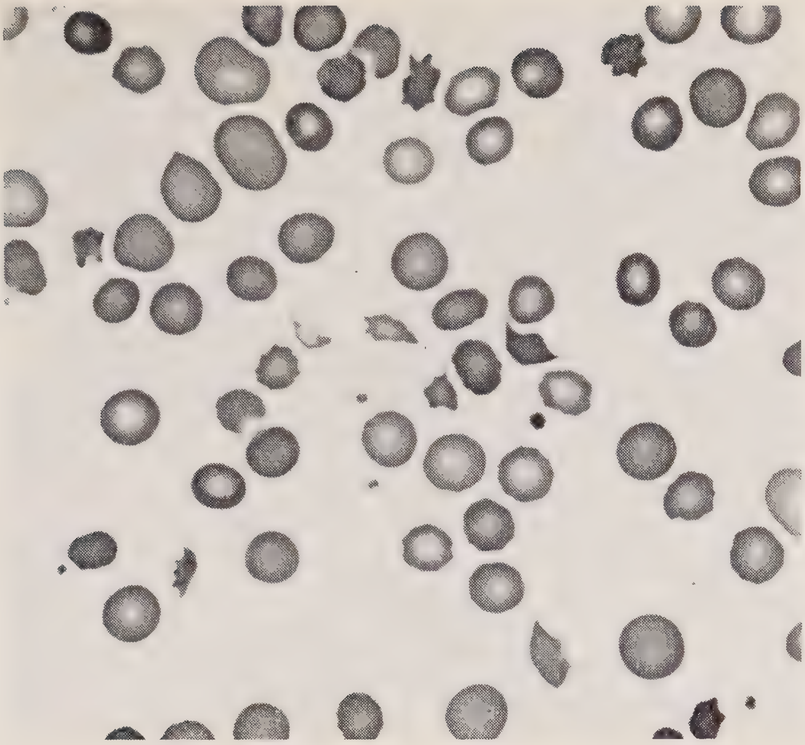

FIG. 244. Peripheral blood film of a patient (A. A.) who developed severe hæmolytic anæmia following the insertion of a Teflon graft for an ostium-primum defect. There is marked anisocytosis and poikilocytosis, and distorted and contracted cells, "burr" cells and cell fragments are present. ×700. (From Sayed *et al.*, 1961.)

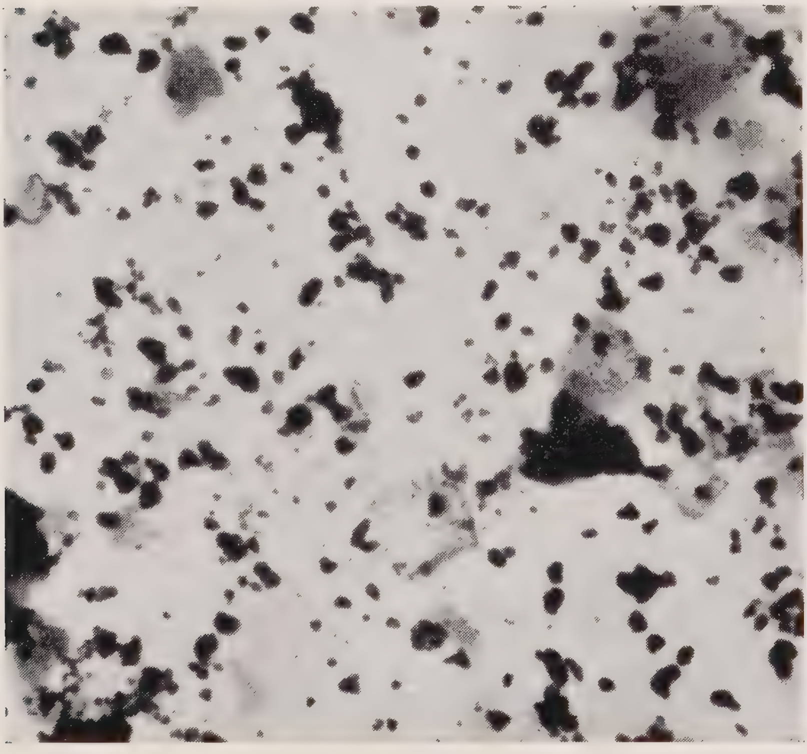

FIG. 245. Photomicrograph of the urinary deposit of a patient (A. A.) who developed a severe hæmolytic anæmia following the insertion of a Teflon patch for an ostium-primum defect. There is marked hæmosiderinuria. Perls's reaction for iron. ×700.

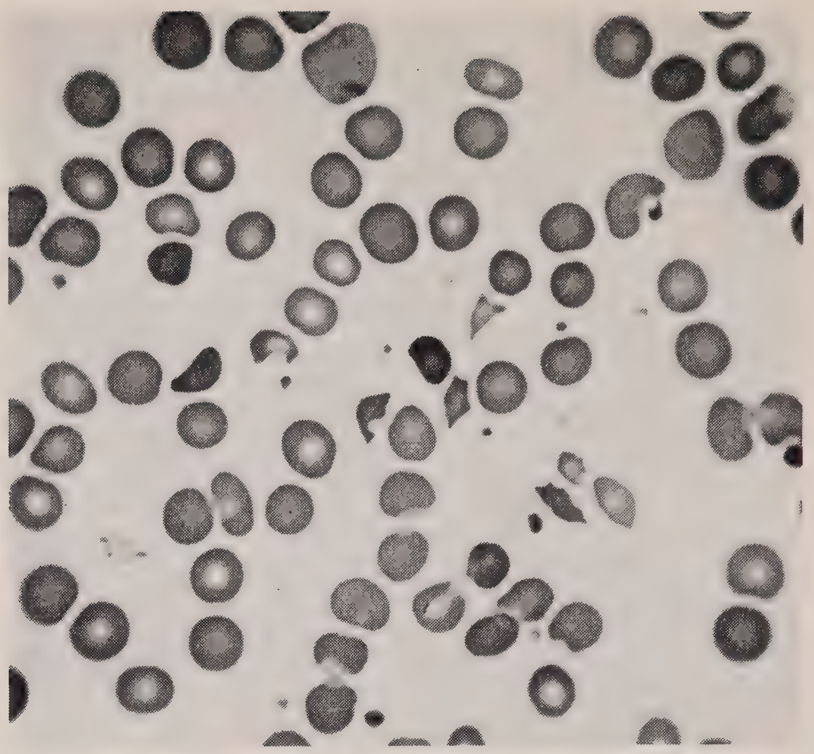

FIG. 246. Photomicrograph of the blood film of a patient (A. W.) who developed a severe hæmolytic anæmia after the insertion of a Starr-Edwards prosthesis for severe calcific stenosis. There is marked anisocytosis and poikilocytosis and many distorted and contracted cells and cell fragments are present. ×700.

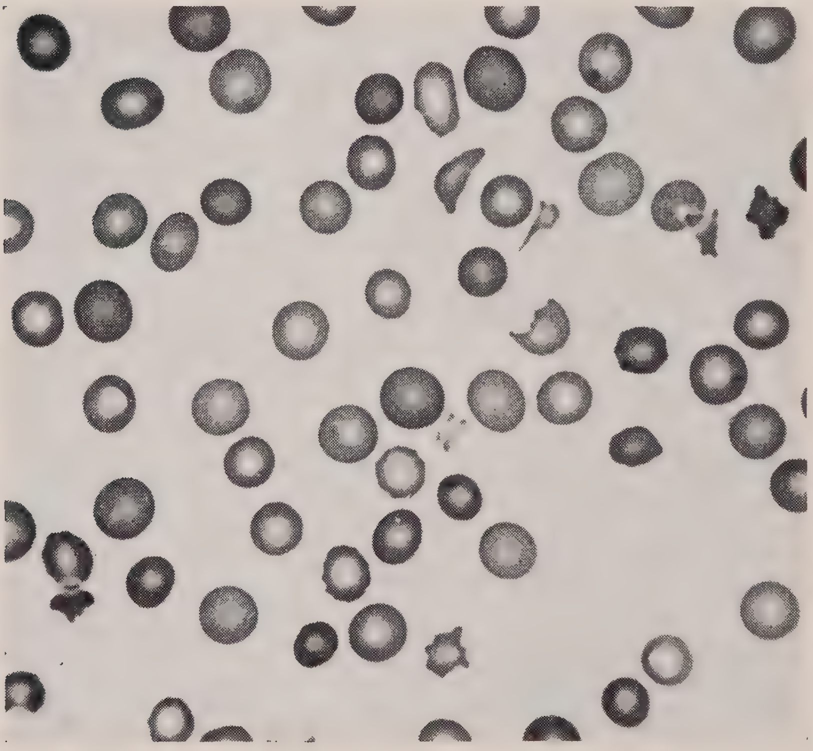

FIG. 247. Photomicrograph of the blood film of a patient (B. B.) who developed a moderately severe hæmolytic anæmia following a mitral-valve replacement. A moderate number of contracted and distorted cells and cell fragments are present. ×700.

Pathogenesis of the Hæmolytic Anæmias following Cardiac Surgery

There is no reason to believe that any of the patients who have suffered from hæmolytic anæmia and have been investigated up to now suffered from any constitutional abnormality of their erythrocytes, and the concept of the hæmolysis being due to alterations in the cells' environment rather than to defects in the cells themselves is strongly supported by the impaired survival of normal transfused erythrocytes (Sayed *et al.*, 1961). There is no evidence, too, in the majority of cases, at least, to support the possibility that the hæmolysis is brought about by an auto-immune mechanism, for the direct antiglobulin test and other tests for abnormal antibodies have usually given negative results. Any resemblance to the known "medical" or spontaneous types of chronic intravascular hæmolysis is superficial only. The blood picture, and other hæmatological and serological findings, when considered together, are quite distinct. Finally, the fact that restoration towards normality at a second cardiac operation has resulted in cessation of hæmolysis (Sayed *et al.*, 1961; Sigler *et al.*, 1963) has established beyond any doubt the cardiac origin of the hæmolysis. The exact mechanism of the hæmolysis is still, however, not quite clear, although there is little doubt but that mechanical factors are involved and that the hæmolysis takes place to a large extent within the blood stream.[1]

Stohlman, Sarnoff, Case and Ness (1956), in their dogs, found that the insertion of a lucite tube into the aorta was not associated with hæmolysis although a lucite ball valve was, and they concluded that the erythrocytes in the latter case were being mechanically damaged between the ball valve and its casing.

Sayed and his co-workers (1961), finding an area of bare Teflon on to which a jet of blood was impinging, concluded that contact between the bare foreign surface was a likely explanation although they did not exclude excessive turbulence as a factor. It is interesting to note that in the very severely affected patient of Verdon, Forrester and Crosby (1963) bare Teflon was again found. It is possible that this lack of endothelial covering is connected with the severity of the hæmolysis in the two cases just discussed. In other ostium-primum repair cases, however, the Teflon septum does appear to have been satisfactorily covered with endothelium and in these patients mechanical stresses caused by turbulence seem likely to have been the sole causal mechanism (Sigler *et al.*, 1963).

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[1] Viner and Frost (1965), in investigating a patient who developed moderately severe hæmolysis after the insertion of three Teflon leaflets in the aortic valve, demonstrated increased splenic sequestration after labelling erythrocytes with ^{51}Cr. Splenectomy did not, however, alleviate the hæmolysis.

The presence or absence of a defect in the endothelial covering to the artificial septum seems likely to depend upon the degree of reflux. Where this is severe the jet of blood may be expected to cause loss of the endothelial covering of the area played upon. Whether lack of an endothelial covering then acts as an additional cause of hæmolysis has not yet been proved.

The role of exposed Teflon as a factor in increasing hæmolysis in endocardial cushion defect repairs is briefly reviewed by Neill (1964). Sears (1965), in a general review on the effect of implants on the blood, concluded that prosthetic materials had not been proved to be directly responsible for hæmolysis.

The role of turbulence and consequent violent pressure changes in producing major degrees of hæmolysis has been underlined by the findings in the aortic-valve prostheses cases, for it now seems clear that when a marked degree of hæmolysis develops a defect in the valve or a partial detachment leading to severe reflux has been present (Stevenson and Baker, 1964; Reed and Dunn, 1964; Gehrmann and Loogen, 1964; DeCesare, Rath and Hufnagel, 1965; Viner and Frost, 1965; Yeh, Ellison and Wright, 1965). Contact with plastic material seems insufficient by itself to cause major degrees of hæmolysis.

Ziperovich and Paley's (1966) recent report of hæmolytic anæmia following an ineffectual plastic operation on the mitral valve shows that severe hæmolysis may develop in this type of case also.

The degree of abnormality to be seen in the blood films of these patients seems to be clearly related to the severity of the hæmolysis, but exactly why and how the erythrocytes become distorted and eventually fragmented remain uncertain. Similar changes cannot be produced by mechanical trauma *in vitro*, at least in short-term experiments. Presumably the abnormalities are the consequence of slowly cumulative physico-chemical changes affecting the cells' surfaces.

The observations of Hjelm and co-workers (1964) and Pirofsky, Sutherland, Starr and Griswold (1965) on the presence of positive direct antiglobulin tests and anti-erythrocyte auto-antibodies in the sera of certain patients after cardiac surgery have already been referred to (p. 960). Most patients, however, certainly do not seem to form auto-antibodies against erythrocytes. The evidence at present suggests, therefore, that mechanical factors are as a rule far more important in the pathogenesis of the hæmolytic anæmias following cardiac surgery than is an auto-immune mechanism.

The Occurrence of Excess Hæmolysis in Unoperated Patients with Cardiac Disease

If it is accepted that mechanical conditions in the heart, particularly severe reflux in a high pressure area, can be a cause of serious intravascular hæmolysis, it seems remarkable that overt hæmolytic anæmia has not been frequently reported as occurring in cardiac abnormalities of natural origin. As already quoted (p. 957), studies using ^{51}Cr have demonstrated that a minor but subclinical degree of excess hæmolysis is frequently present in patients who have severe disease of the aortic valve. In fact, a very small number of cases of overt hæmolytic anæmia have been reported within the last year or so (see below) and no doubt now that physicians have been alerted to the possibility the occurrence will prove to be not so rare as it appears to be at the time of writing. It may perhaps be added that the author is not aware of a single proved case at Hammersmith Hospital within a 20-year period.

In the case discussed by Dameshek and others (1964) overt hæmolytic anæmia, associated with distorted (helmet) erythrocytes in the blood film, was present in a patient who had severe aortic and mitral stenosis and insufficiency and also tricuspid stenosis. Numerous calcified excrescences were present on the mitral valve and the free margins of the aortic valves were also calcified. Renal siderosis was found at necropsy and there seems little doubt but that the patient had suffered from intravascular hæmolysis. But whether this was of cardiac origin or not could not be established. There did not, however, seem to be a satisfactory alternative explanation.

Two further patients with valvular heart disease in which excess hæmolysis appeared likely to have a mechanical (*i.e.*, intra-cardiac) origin have been more recently described. Westring's (1966) patient had probably a mild hæmolytic anæmia pre-operatively and a more severe one after the insertion of a Starr-Edwards valve which subsequently tore along the suture line. (Westring also studied 12 other patients with aortic-valve disease and found that three had shortened ^{51}Cr $T\frac{1}{2}$ times, although only one showed any other signs of increased hæmolysis.) Miller and co-workers' (1966) patient had rheumatic disease of both aortic and mitral valves and what appeared to be a mild hæmolytic anæmia (hæmoglobin, 11·4 g. per 100 ml.; reticulocytes, 6·3%). A ^{51}Cr study gave, however, a normal result when the patient was at rest. When he undertook, however, regular daily exercise—thus increasing his cardiac output—the ^{51}Cr $T\frac{1}{2}$ fell to about 17 days and the hæmoglobin fell by 1·5 g. per 100 ml. (Miller and his colleagues also investigated 20 other patients with valvular disease but found no evidence of intravascular hæmolysis in any of them.)

Another type of association was described by Nikkilä and Vuopio (1964): their patient had a left atrial myxoma and also hæmolytic anæmia and a severe grade of thrombocytopenia which, it was suggested, might have been the consequence of abnormal flow conditions in the left side of the heart.

MARCH HÆMOGLOBINURIA

March or exertional hæmoglobinuria is one of the three classical types of hæmoglobinuria described in the 19th century. Fleischer in 1881 under the title "Ueber eine neue Form von Hæmoglobinurie beim Menschen" reported its occurrence in a soldier after a strenuous (Feld) march. Many similar cases have since been described and there is in consequence an extensive literature.

The occurrence of the hæmoglobinuria is characteristically associated with exercise (see below); it is not related to sleep or cold or to disease of any sort. The passage of dark urine lasts as a rule only a matter of a few hours, and the loss of hæmoglobin and the frequency of attacks are generally insufficient to cause any appreciable degree of anæmia. The patients, too, do not as a rule suffer from any symptoms other than the hæmoglobinuria: in particular, chills and fever are usually absent. Hæmosiderin is present in the urine in subjects who have frequent attacks and in rare instances the spleen is palpably enlarged. When attacks are frequent a slight rise in serum bilirubin may be found and slight reticulocytosis; the serum-haptoglobin concentration may be reduced (Davidson, 1964).

Many studies have been carried out on the patients' red cells without demonstrating any clear abnormality. Their morphology appears to be normal (? invariably) and mechanical fragility, in particular, is normal (Gilligan and Blumgart, 1941; Hobbs, 1944).

Although originally observed in soldiers after marching, similar degrees of hæmoglobinuria have been provoked in other cases by running or even walking; significantly, hæmoglobinuria has not been observed after many other types of strenuous exercise such as swimming or exercise (to the point of exhaustion) on a stationary bicycle. The story of the unravelling of the cause of march hæmoglobinuria is a fascinating one. It is a chastening one, too, for it has taken more than 80 years to arrive at what appears to be the correct (and simple) explanation: namely, that hæmoglobinuria follows intravascular hæmolysis brought about by mechanical trauma to the blood within the feet as the result of marching, walking or running heavily on a hard surface (Davidson, 1964).

Literature on March Hæmoglobinuria

Some of the more important publications on march hæmoglobinuria will be briefly referred to. Fleischer's (1881) original description has already been mentioned. Dickinson's (1894) early account is an interesting one. He described three cases (one of which he had personally observed): in two instances the hæmoglobinuria followed running; in one it had been brought on by a game of tennis. The running ground attendant was quoted as stating that "it is not very uncommon for the urine to appear bloody after a severe run, especially at the beginning of training." Dickinson, who regarded the hæmolysis as being brought about by muscular exercise, concluded that it was "perhaps an exaggeration of the blood-destruction which is the normal result of exercise."

Feigl's (1916) account is also important as he reported that traces of hæmoglobin and/or hæmatin might frequently be found in the serum of soldiers after marching (*e.g.*, in 15 out of 27 soldiers) and that traces of blood pigment might often be found in the urine, too. This paper thus suggested that overt march hæmoglobinuria might in fact be an exaggeration of a phenomenon which was virtually physiological. Fisher and Bernstein (1940) carried out studies on a Marathon runner; they noticed that he ran in a rather unconventional way, leaning a little backwards, and they stressed the importance of posture. They made the point that the disorder occurred predominantly in young men.

Gilligan and Blumgart (1941) published an account of three further cases and surveyed the literature in an extensive review. They found accounts of 40 cases; all were men, within the age range 16 to 35 years. With few exceptions the attacks cleared up within a few months to 2 years. They showed that the hæmoglobinuria developed secondarily to hæmoglobinæmia, that the hæmoglobinæmia was maximal immediately after the exertion and that the relationship between the level of plasma hæmoglobin and the presence of hæmoglobin in the urine was similar to that occurring in other types of intravascular hæmolysis; they also noted when attacks were frequent that hæmoglobin might appear in the urine at relatively low plasma hæmoglobin concentrations.

Gilligan and Blumgart also gave many references to papers in which the relationship between the type of exercise and the resultant hæmoglobinuria was discussed. From the earliest reports it had been noted that walking or marching might produce hæmoglobinuria whilst other types of strenuous exertion did not; in particular, they pointed out that while walking in the normal upright position might provoke hæmoglobinuria, walking in a kyphotic position failed to do so (Porges and Strisower, 1919). Gilligan and Blumgart investigated this problem in detail themselves and showed conclusively that hæmoglobinæmia and consequent hæmoglobinuria did not occur when one of their patients exercised in a kyphotic position on a bicycle ergometer.

Gilligan and Blumgart reviewed the various theories extant at the time of their review—namely, hæmolysis in the kidneys or in the spleen, hæmolysis as the result of upright posture or due to hyperventilation—and concluded that none of these theories provided a tenable explanation. They found, too, that there was no relationship between the hæmoglobinuria and orthostatic albuminuria. They concluded (with Feigl, 1916) that "physiological" hæmoglobinæmia and hæmoglobinuria occur in healthy individuals after marches and runs provided the exertion is intense and of considerable duration, and that in march hæmoglobinuria the same mechanism operates, the difference being that the hæmolysis then results from lesser degrees of exertion. The actual cause of march hæmoglobinuria they attributed to some, as yet undefined, mechanical or postural abnormality.

Gilligan and Altschule's (1950) paper is notable in being the first in which march hæmoglobinuria was reported in a woman (against 76 cases in men reported in the literature up to that time); in this particular woman walking half a mile in 10 minutes was sufficient to bring on an attack.

Further details of the extent to which "physiological" degrees of intravascular hæmolysis might follow strenuous exercise were published by Gilligan, Altschule and Katersky (1943). Significant degrees of hæmoglobinæmia were found to occur in many athletes who ran 2·6 miles, and of 22 men who ran the Marathon distance (26·2 miles) 18 developed hæmoglobinæmia (and four of them had hæmoglobinuria).

The effect of exercise on the blood picture of dogs has been studied by a number of workers and Broun (1922, 1923) obtained evidence consistent with the theory that exercise in a treadmill led to hæmolysis, particularly in dogs exercised after prolonged confinement—but the mechanism of the probable hæmolysis was not determined. Similar results have been more recently reported by Hiramatsu (1960).

More Recent References. Pare and Sandler (1954) reported that the urine of sufferers from march hæmoglobinuria contains abnormal amounts of amino-acids such as cysteine and β-amino-isobutyric acid. The significance of these observations is as yet unknown.

Martin and Kilian (1959) reviewed the literature once more and studied a single patient in detail. All attempts to demonstrate a specific erythrocyte abnormality failed and no abnormal antibodies could be demonstrated. Serum complement was unaltered. However, a decrease in properdin activity was found and erythrocytes taken immediately after marching showed increased autohæmolysis in normal serum; this appeared to be dependent on the presence of properdin in the serum. The significance of these observations, too, is unknown. Martin and Kilian attributed the hæmolysis to a "transitory acquired erythropathia" of unknown origin.

Flatmark's (1963) report is interesting because his patient was a man of 54 who had not had hæmoglobinuria before. Like Martin and Kilian he also observed increased hæmolysis of his patient's erythrocytes, taken after exercise, when incubated in normal serum.

Schlatter and Forster (1965) discussed the differentiation between "sport" (march) hæmoglobinuria and myoglobinuria, which also can occur after physical exercise. In the latter condition increases in muscle enzymes (glutamic-oxaloacetic transaminase, lactic dehydrogenase and creatine phosphokinase) can be demonstrated in serum: in march hæmoglobinuria such increases are not found. Lundquist (1965) reported in one case a rise in blood sugar to 160 mg. per 100 ml. which ran parallel but slightly preceded the rise in plasma hæmoglobin.

Balcerzak, Wheby, Goler and Crosby (1966) carried out careful exercise tests in a young man who had experienced march hæmoglobinuria and showed that 125 minutes' exercise in a treadmill resulted in a rise in plasma hæmoglobin, a small amount of hæmoglobinuria, diminution in urine flow, proteinuria and the passage of numerous casts. The hæmoglobin-binding capacity of the plasma was abnormally low, but it rose after rest in bed. Balcerzak and his co-workers concluded that in this particular man even a normal degree of exercise produced a minor degree of hæmolysis.

March Hæmoglobinuria—The Explanation

As mentioned earlier, Davidson (1964) suggested, and provided supporting evidence, that the intravascular hæmolysis of exertional hæmoglobinuria is brought about by mechanical trauma to the blood during its passage through the feet. Davidson, who investigated three patients, noticed in the first two that although hæmoglobinuria occurred after runs on hard roadways, both runners could run more than 5 miles on a cinder or grass track without overt hæmoglobinuria developing. In his third patient hæmoglobinuria only occurred after running on a hard wooden floor. Davidson, appreciating the possible significance of the surface on which running was carried out, then conducted some experiments with blood in plastic tubes inserted into the patients' running shoes. He found that such blood regularly sustained substantial amounts of hæmolysis if the patients ran in the shoes. He found, too, with the two patients he studied, that each of them hæmolysed more blood than did control subjects, that the blood of patients and controls was equally sensitive to traumatic lysis under these experimental conditions and that more lysis occurred when the test runs were carried out on a hard road surface than on grass. Davidson also compared the plasma-hæmoglobin levels resulting from running on a road-surface and on grass, respectively, and found that only very small rises occurred on grass compared with the substantial rises which followed the running on the road. The patient described as Case I, who had a heavy and rather stamping stride, produced the highest plasma-hæmoglobin level. Davidson also reported that since this same patient had taken to wearing resilient insoles in his running shoes there had been no recurrence of hæmoglobinuria despite vigorous training.

Finally, as pointed out by Davidson, mention should be made of the paper of Attlee (1937) who observed in two public schoolboys that, although strenuous exercise on grass did not result in symptoms, hæmoglobinuria followed running on a hard road. Attlee realized that his observation was unlikely to be coincidental but he did not appreciate its significance.

Davidson's study appears to have solved the 80-year-old problem of march hæmoglobinuria! Certainly, most of the hitherto recorded facts fit in well with Davidson's conclusions, and they provide a logical explanation for the quite common incidence of march hæmoglobinuria in service recruits at the time of their preliminary training. However, whether all the phenomena of march hæmoglobinuria can be explained on the above-described basis seems uncertain at the time of writing; the occurrence, for instance, of march hæmoglobinuria for the first time in a man of 54, as described by Flatmark (1963), is not easy to explain.

That mechanical trauma to erythrocytes can cause hæmolysis *in vivo* as the result of contact between the feet and a hard surface is perhaps not too unexpected when it is recalled that major degrees of hæmolysis can be brought about by mechanical forces following cardiac operations (see p. 963). In the latter type of hæmolytic anæmia abnormalities of erythrocyte morphology are conspicuous; but except for the report of Watson and Fischer (1935), who remarked on poikilocytes and "four-leaf clover" cells, erythrocyte abnormalities do not seem to have been noted in march hæmoglobinuria.

Davidson's (1964) excellent work has been confirmed by subsequent studies. Buckle (1965), in describing the case of a steeple-chaser who had developed hæmoglobinuria following running on built-up roads (but seldom as the result of cross-country running), found that the use of sorbo-rubber insoles reduced the incidence of hæmolytic episodes. Crosby (1965), in discussing the role of trauma to the blood in the causation of march hæmoglobinuria, pointed out that Ensor and Barrett had described as long ago as 1903 a patient suffering from dementia præcox who would slap his forehead with extreme violence for 20–30 minutes on end, each blow being accompanied "by a sickening thud." This remarkable activity was regularly followed by an episode of hæmoglobinuria. The discussion into the mechanism of hæmolysis—whether due to effusion of blood into the areolar tissue overlying the skull and subsequent lysis due to the action of "hæmolysins" or due to the trauma itself—makes interesting reading.

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